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MENDELSKA SÄLLSKAPET

Lund den 15 november 1960

ON THE ORIGIN OF CULTIVATED LETTUCE

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(Received April 5th, 1960)

IN connection with cytological and genetical investigations within the *serriola* group of *Lactuca*, results of which have been published in earlier papers (LINDQVIST, 1956, 1958, 1960 a, 1960 b), material was obtained of a number of *Lactuca* forms, the identity of which was at first obscure. They had been given various names, but as the investigations progressed it became clear that they should be considered as primitive types of *L. sativa*, although none of them had been received under that name. The purpose of this paper is to provide taxonomic and cytogenetic evidence in support of this opinion and to consider the bearing of the existence of these forms on the theories about the origin of cultivated lettuce.

I. TAXONOMY OF THE SERRIOLA SECTION

1. Delimitation of species

The genus *Lactuca* belongs to the tribe *Cichoriae* of the *Compositae* family. It embraces about a hundred species with the haploid chromosome numbers 8, 9 or 17. These species are divided into a number of sections, one of which is the *Scariola* section, which includes the *serriola* group (THOMPSON, WHITAKER and KOSAR, 1941; THOMPSON, 1943), consisting of the four 9-chromosome species *L. saligna*, *L. virosa*, *L. serriola* and *L. sativa*. THOMPSON, WHITAKER and KOSAR (*l.c.*) included in this group also *L. altaica* FISCH. and MEY. which will be discussed later in this paper.

The above mentioned authors found that the *serriola* group occupies an isolated position within the genus *Lactuca*. Among numerous attempted crosses with other species only one combination was successful, namely *L. graminifolia*×*virosa*, producing one partly fertile F_1-hybrid. It is, of course, not impossible that other species, not yet investigated

cytogenetically, bridge the gap between the *serriola* group and other species groups.

The four species of the *serriola* group may be identified with the aid of the following key:

Plant biennial; achenes purplish black, wing-margined, rugose but not spiculate or setose ..... *L. virosa*

Plant annual or winter annual; achenes brown, grey or white, not wing-margined, not or very slightly rugose, but more or less spiculate or setose towards the apex.

Panicle spike-like; beak of pappus longer than the achenes; achenes spiculate, but not setose ..... *L. saligna*

Panicle open; beak of pappus same length as the achenes; achenes more or less setose but not spiculate.

Involucre reflexed when achenes ripe, panicle pyramidal ..... *L. serriola*

Involucre erect when achenes ripe, panicle flat-topped ..... *L. sativa*

With the exception of the involucre character used to separate *L. serriola* and *L. sativa*, these key characters are well established and generally accepted (*cf.* GRENIER and GODRON, 1850; BISCHOFF, 1851; HEGI, 1929; STEBBINS, 1939). The involucre characters have been found to provide a very reliable means of separating *L. sativa* and *L. serriola*. In the course of this study a large number of cultivated lettuce varieties have been examined with regard to involucre characters and all were found to have the erect involucre (*cf.* MC COLLUM, 1953; WHITAKER and MC COLLUM, 1954). This character was not found on any specimen of a very large amount of material of *L. serriola*, which was studied partly in cultivation, partly in natural populations and partly on herbarium specimens. If the character exists in this species it can be only as a very rare exception.

Most authors agree that *L. virosa* and *L. saligna* are species, which are well separated from *L. serriola* and *L. sativa*. BENTHAM and HOOKER (1947), however, point out that forms exist which are intermediate between *L. saligna* and *L. serriola*. Specimens agreeing with *L. saligna* with regard to general habit and with regard to length relation between pappus and achenes, but having the open panicle typical of *L. serriola*, have been encountered by the writer in various herbaria. As an example may be mentioned a specimen with the name '*L. Scariola* L, var. *adulterana* GR. et GODR.' at the Botanical Museum, Lund, Sweden.

There is some controversy as to whether *L. sativa* should be considered as a separate species or as a variety of *L. serriola*. It was given specific rank by LINNEAEUS (1753) and this has been accepted by many authors, *e.g.* HEGI (1929), and GRENIER and GODRON (1850). The

opinion that *L. sativa* is only a cultivated form of *L. serriola* was held by BISCHOFF (1851) and was shared by BOISSIER (1875), HOOKER (1875—1897), FIORI (1925—1929) and others.

Cytogenetic investigations by THOMPSON, WHITAKER and KOSAR (1941), THOMPSON (1943) and LINDQVIST (1960 a) have given a picture of the relationships between the species of the *serriola* group, which agrees very well with that based on taxonomic evidence. There are clear differences between *L. virosa* and the other species with regard to chromosome morphology, and gene exchange appears to be almost impossible. Strong sterility barriers exist between *L. saligna* on one side and *L. serriola* and *L. sativa* on the other, and also minor differences in chromosome morphology, but gene exchange is still possible. *L. sativa* and *L. serriola*, finally, have chromosomes which appear to be identical morphologically and largely homologous, and there are no obstacles to gene flow between these two species. In biosystematic terminology the *serriola* group thus consists of three cenospecies, namely *L. virosa*, *L. saligna* and *L.sativa-serriola*.

There is no indication of natural hybridization between *L. virosa* and *L. serriola* or *L. saligna* (*cf.* STEBBINS, 1939), but some evidence of the occurrence of spontaneous hybrids between *L. saligna* and *L. sativa-serriola* (LINDQVIST, 1960 a), and fertile derivatives have in experiments been obtained from hybrids between *L. saligna* and *L. sativa*, in spite of a very high degree of sterility in F_1. It is thus possible that some exchange of genes takes place in nature between *L. saligna* and *L. sativa-serriola*, and that the intermediate forms mentioned above are of hybrid origin. Spontaneous hybridization takes place occasionally between *L. sativa* and *L. serriola*. OWNBEY (1949) found hybrids with *L. sativa* in a natural population of *L. serriola* and the hybrid nature of these plants was confirmed by MC COLLUM (1953). Other cases have been reported by PAMMEL (1918) and by BOHN and WHITAKER (1951), who state that the occurrence of a few natural hybrids with *L. serriola* is not uncommon in commercial lettuce fields. The spontaneous hybrid described by NAUDIN (1875) as *L. sativa*×*virosa* was in all probability another example (*cf.* LINDQVIST, 1960 a, p. 132). No hybrids have been observed by the writer in natural populations.

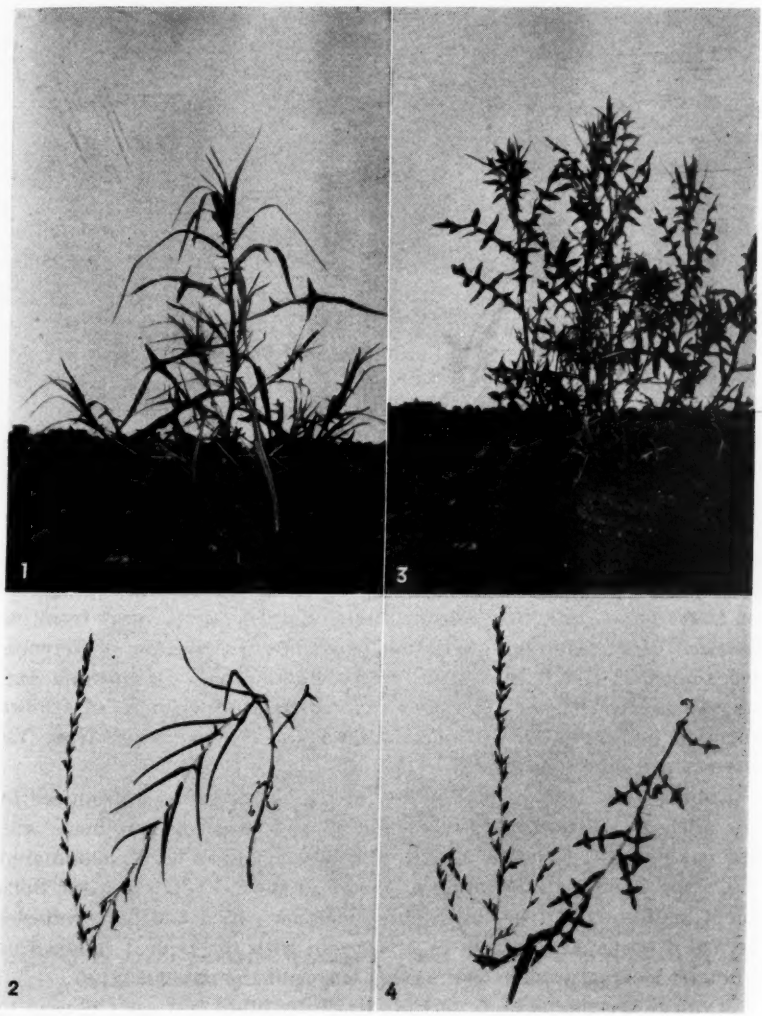
The three wild species all occur as weeds of road sides and waste places, but *L. virosa* is also found in half-wild habitats, such as sand dunes and rock crevices. *L. serriola* is by far the most common and 'aggressive' of the three. They all originate from the Old World, where they have their main distribution centre round the Mediterranean. With

increasing world communications they, particularly *L. serriola*, have spread to all parts of the world. In U.S.A. this species, which was first recorded in 1863 (PAMMEL, 1894) is a very common weed, which is much more troublesome than anywhere in Europe, whereas *L. saligna* and *L. virosa* have a very limited distribution in U.S.A. *L. virosa* is, in various parts of Europe, cultivated on a small scale for the sake of its milk juice, which has narcotic properties and which in dried form finds a use in medicin under the name of Lactucarium.

2. Variation within the species

L. saligna. — The typical form of this species has long, narrow, pointed almost linear leaves. The basal leaves are pinnatifid, but leaves on the seed stalk almost entire. A form with somewhat broader, clearly lobed leaves on the seed stalk is var. *runcinata* (GRENIER and GODRON, 1850; HAUSSKNECHT, 1895), which by GRENIER and GODRON (*l.c.*) was suspected to be a hybrid between *L. serriola* and *L. saligna.* Lines which fit the description of the two forms have been studied cytogenetically by the writer (LINDQVIST, 1960 a) and where found to be very similar, but they differed with respect to viability of F_1-hybrids with *L. sativa.* Specimens of these forms are illustrated in Figs. 1—4. A prickly form (f. *Ruppiana* WALLR.) and a non-prickly form (f. *Wallrothi* SPRENG) are mentioned by HEGI. All specimens of *L. saligna* examined by the writer had prickles along the dorsal side of the midrib of the leaf, but these prickles were fewer and softer than in *L. serriola*, and they did not continue down on to the stem, which is the rule in the latter species. This spination is similar to that produced by the allele sp_1 in the primitive *L. sativa* form Altaica (LINDQVIST, 1960 b). With regard to anthocyanin pigmentation, typical specimens of *L. saligna* belong to the tinged type (THOMPSON, 1938) and no variations have been reported.

L. virosa. — Within this species there is variation with regard to leaf form, spination and anthocyanin pigmentation. Non-lobed forms exist and forms with lobing similar to that of *L. serriola.* Some forms are completely non-prickly, others have degrees of prickliness similar to that produced by the alleles sp_2 or *Sp* in *L. sativa-serriola.* Anthocyanin pigmentation may be lacking or it may be present in varying degrees, with or without spots on the leaves. All specimens examined in cultivation, in natural habitats or as herbarium specimens had broad leaves with blunt apices. Specimens of *L. virosa* are illustrated in Figs. 5—7.



Figs. 1—4. *L. saligna*, cultivated specimens. — Figs. 1—2. Line Sal.C. Figs. 3—4. Sal.R (f. *runcinata*). Figs. 1 and 3. Growing plants. Figs. 2 and 4. Pressed specimens.

Variations such as those described here appear in botanical literature under various names. A green-leaved form (presumably lacking anthocyanin) has been referred to as *L. virosa* f. *flavida* JORD. (GRENIER and

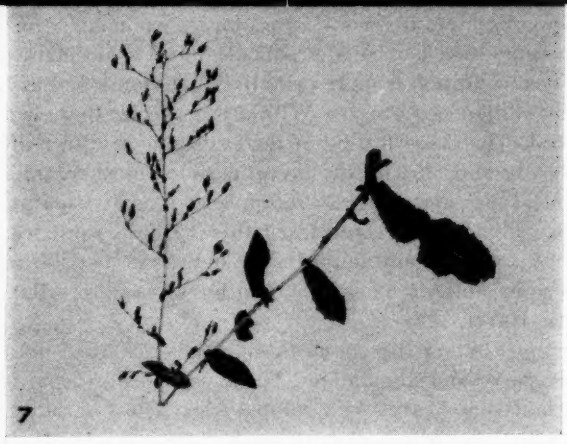
GODRON, 1850), and forms with spotted leaves as *L. virosa* f. *maculata* (DE CANDOLLE, 1838) and *L. virosa* var. *cruenta* NOBIS. A form which is very close to *L. virosa* was described by BOISSIER (1839—1845) under the name *L. livida* BOISS. and REUT. It differed by having more deeply lobed leaves and anthocyanin pigmentation of a violet shade in the whole plant.

L. serriola. — The typical form of this species has lobed leaves with blunt apex, prickles on stem and leaves and anthocyanin pigmentation of the tinged type. A non-lobed form, f. *integrifolia* BOGENHARD occurs more or less frequently throughout the distribution area of the species, and mixed populations of the typical and the non-lobed forms are not uncommon. A non-lobed, non-prickly form from Val d'Aosta, Italy, was described by ALLIONI (1786), who gave it specific rank under the name *L. augustana* ALL., but it has been included in f. *integrifolia* by many authors, *e.g.* BISCHOFF (1851). The writer had had an opportunity of studying populations of *L. serriola* in Val d'Aosta, and has found that all four combinations of the characters lobed, non-lobed, prickly and non-prickly occur together there. Lobed, non-prickly forms have been seen also in other parts of Italy. Progenies of non-lobed, non-prickly plants from Val d'Aosta have been grown side by side with progenies of *L. serriola* f. *integrifolia* from England and France. Apart from the absence of prickles in the Italian progenies no essential differences were noted. There is thus every reason to consider *L. augustana* ALL. merely as a variety of *L. serriola* f. *integrifolia*. Specimens of a lobed form, a non-lobed form from England and *L. augustana* from Val d'Aosta are shown in Figs. 8—11.

Lobing in *L. serriola* is normally of the runcinate type produced by the allele U_{lob} (LINDQVIST, 1958), but some herbarium specimens with the oakleaved type (allele U_{oak}; LINDQVIST *l.c.*) have been encountered, *e.g.* a specimen with the name *L. plicata* in Herb. F. NEGRI at the Botanical Institute at Turin, Italy. This specimen which had been collected in Val d'Aosta, was a form of *L. serriola* with the typical reflexed involucre, lacking prickles and having leaves of the oakleaf type.

Typical specimens of *L. serriola* have the blunt leaf apex caused by the recessive gene *p* (LINDQVIST, 1960 b) and only one exception to this rule is known, namely a form from Idaho, U.S.A., used by MC COLLUM (1953) in his cytogenetic study. It is obvious from the description and illustration in MC COLLUM's paper that this form has the leaf type resulting from interaction of the genes U_{lob} and *P* (LINDQVIST, 1960 b).

All specimens of *L. serriola* examined by the writer have had the



Figs. 5—7. *L. virosa*, cultivated specimens. — Figs. 5 and 6. 1st year plants of two different lines. Fig. 7. Line Vir.L; pressed twig of flowering plant.

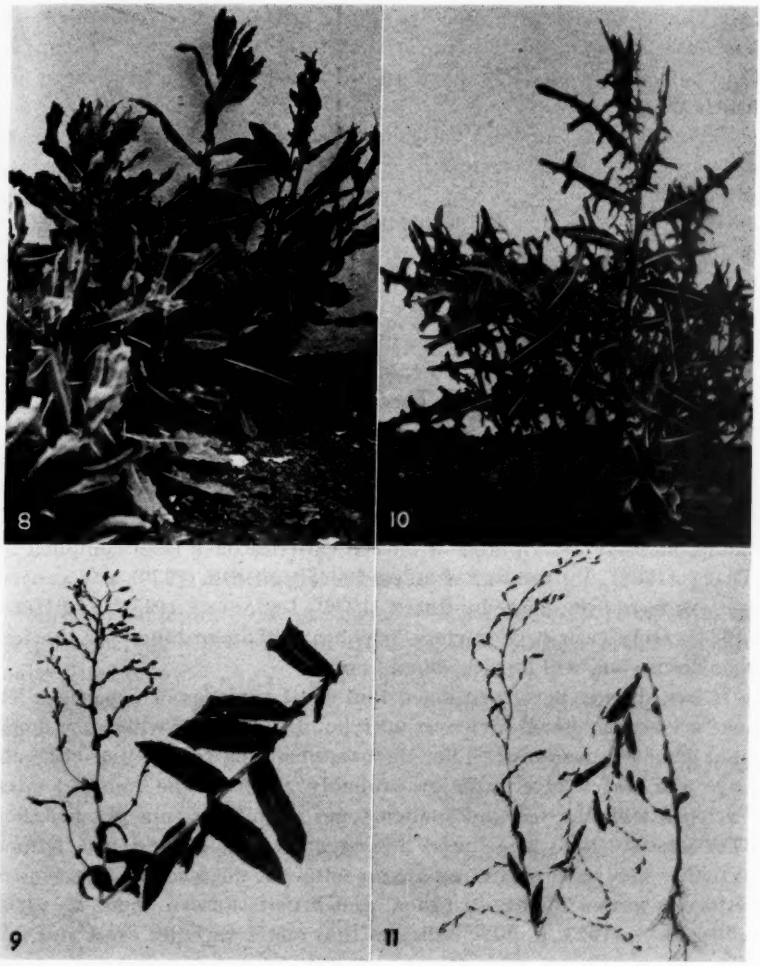
tinged type of anthocyanin pigmentation produced by the allele *r* in the *R-r'-r* series (THOMPSON, 1938) *i.e.* anthocyanin is present only at the leaf margins, but there are no spots or red coloured areas on the lamina, and there is no reliable evidence that any of the other alleles occur in this species. Form with spotted leaves are, however, mentioned by BISCHOFF (1851), namely *L. scariola* var. β LINN. and *L. scariola* β *ma-*

culosa DE C. Both LINNAEUS (1753) and DE CANDOLLE (1838) refer to a description and illustration by PAUL HERMANN (1705) of a form called *Lactuca sylvestris Italica, costa spinosa, sanguineus maculis adspersa.* The illustration agrees, however, better with *L. virosa* than with *L. serriola.* The form is described as having '*semina nigra*' which indicates that it really belongs to *L. virosa.*

The very exceptional occurrence of the alleles U_{lob}, *P* and possibly *R* or *r'* in *L. serriola* is probably a consequence of natural hybridization with forms of *L. sativa* carrying these alleles. MC COLLUM (1953) states that his Idaho form, which had pointed leaf apex, also had bigger achenes than the other *L. serriola* form with which he was working, and that the ligules of the Idaho form had a much deeper yellow colour. Both these characters might well have been introduced from *L. sativa* (see page 331 below concerning size of achenes). MC COLLUM (*l.c.*) discusses the possibility that the non-lobed form of *L. serriola* is a derivative of a hybrid between lobed *L. serriola* and non-lobed *L. sativa.* This possibility suggests itself, particularly with regard to *L. augustana* ALL., which has also the non-prickly condition in common with *L. sativa.* However, as pointed out by MC COLLUM there is no certain evidence that the lobed condition is more primitive than non-lobed in *L. serriola.*

L. sativa, cultivated forms. — Within this species there is a tremendous variation. The classification of the multitude of different cultivated varieties has been dealt with by several authors, most recently by HELM (1954). Four types are generally recognized, namely asparagus lettuce, cos lettuce, cabbage lettuce and leaf lettuce. Among these, cos and cabbage lettuce are the most important. They form two distinct groups which by some authors are believed to have separate origin (STURTEVANT, 1886; HELM, 1954).

In *asparagus lettuce* the young fleshy stems are used, not the leaves, which are coarse and unpalatable. Some varieties of asparagus lettuce have long lanceolate leaves with pointed apex. These varieties have been confused with ALLIONI's *L. augustana* (STURTEVANT, 1886; HELM, 1954, p. 100), and they often appear under the name *L. augustana* or *L. angustana.* The true *L. augustana* ALL., however, has leaves with the blunt apex typical of *L. serriola*, and there is, in fact, little similarity between this form and lanceolate-leaved asparagus lettuce. Another type of asparagus lettuce is represented by Chinese varieties of which the most well-known is Burpee's Celtuce. These varieties are characterized by a light grey leaf colour not found in any other lettuce varieties. In leaf shape they resemble the next group of varieties.



Figs. 8—11. *L. serriola.* — Figs. 8 and 9. Line Sc.H. Fig. 10. Sc.J. Fig. 11. Sc.aug.All. Figs. 8 and 10. Growing plants, Figs. 9 and 11. Pressed specimens. Figs. 8—10. Cultivated specimens, Fig. 11. Specimen collected on natural habitat.

The *cos lettuce* varieties have oblong, upright leaves with a prominent midrib, running almost to the apex which is rounded. The lamina is rigid and rather coarse in texture. Most cos varieties grown to-day are self-folding, forming loose oblong hearts. In Egypt a form with oblong

leaves, known as f. *aegyptiaca* is widely grown. Material of this form has been obtained by the courtesy of Prof. V. LAURENT TÄCKHOLM, Cairo. It can be described as a primitive cos variety which forms no hearts whatsoever.

Cabbage lettuce varieties have broad, almost orbicular leaves which are more prostrate than those of cos lettuce. The midrib branches into smaller veins before reaching the apex of the lamina. Cabbage lettuce varieties have more tender leaves than cos lettuce, and they form more or less firm hearts. These develop in a different way to those of cos lettuce, and the process is controlled by different genes (LINDQVIST, 1960 b).

The *leaf lettuce* varieties are with regard to leaf texture and appearance of the midrib similar to cabbage lettuce, but differ in their inability to form hearts. They constitute a very heterogeneous group, which includes some extremely curly and fringed varieties (*e.g.* Grand Rapids).

There is much disparity between varieties of the four types with regard to a number of morphological and physiological characters. Comprehensive descriptions of lettuce varieties have been compiled by TRACY (1904), VILMORIN-ANDRIEUX (1920), MORSE (1930) and general surveys have been made by BRIAN (1936), LENANDER (1951) and HELM (1954). Only certain characters, which are of importance for the further discussion, will be considered here.

It has already been mentioned that some varieties of asparagus lettuce are characterized by leaves with pointed apex for which the dominant gene *P* is responsible, but the overwhelming majority of cos, cabbage and leaf lettuce varieties, probably all, have the rounded apex. Varieties with the red and spotted types of anthocyanin pigmentation (THOMPSON, 1938) are found among cos, cabbage and leaf lettuce. Whether they exist also in asparagus lettuce is not known. This type of lettuce is grown mainly in China, and little is known about its variability. BOIS (1927, p. 301) indicates that many varieties exist and that they differ, among other things, in anthocyanin pigmentation. Cultivated lettuce varieties normally have non-lobed leaves, but oakleaved and lobed varieties occur in the asparagus and leaf lettuce groups (TRACY, 1904; BOIS, 1927). Specimens of three varieties of *L. sativa* are illustrated in Figs. 12—14, mainly for comparison with the primitive forms to be described below.

Figs. 12—13. *L. sativa*, growing plants. — Fig. 12. Line K.driv. Fig. *13*. Line Maik.

3. Primitive forms of L. sativa

Source of material. — These forms which have been referred to in the introduction, were received from botanic gardens under various names listed below.

Designation	Origin	Name under which received
Altaica	Kew, England	*L. altaica* FISCH. and MEY.
LC	Copenhagen, Denmark	*L. livida* BOISS. et REUT.
SL	Lund, Sweden	*L. saligna*
SP	Palermo, Italy	*L. sagittata*
„	Pisa, Italy	„ „
„	Tabor, Czechoslovakia	*L. augustana* REICHB.
SB	Brünn, „	*L. virosa*
SM	Modena, Italy	*L. dregeana* D.C.
SN	Nancy, France	*L. virosa*
ST	Turin, Italy	*L. serriola*

Cytogenetic data. — With the exception of the lines called SN and ST, these forms have all been studied cytogenetically (LINDQVIST, 1960 a). The results showed that they are only remotely related to *L. saligna* and *L. virosa*, but that they agree with *L. sativa* and *L. serriola* in chromosome morphology and that hybrids with these species have normal meiosis and are highly fertile. They must therfore be considered as belonging to the *L. sativa-serriola* ecospecies.

Designation. — The fact that these forms were received under such a variety of names, shows that they have not been recognized as an entity in spite of their very close relationship. Most of these names cannot with justification be used for forms of the *L. sativa-serriola* complex. The names *L. saligna* and *L. virosa* must obviously be ruled out. *L. livida* was used by BOISSIER (1839—1845) for a form very close to *L. virosa* (*cf.* p. 324). Judging from BOISSIER's description, the only similarity between this form and the line LC is in the anthocyanin pigmentation. The names *L. sagittata* and *L. augustana* REICHB. are synonyms of *L. quercina* L subsp. *Chaixii* VILL., which is entirely different from these forms. DE CANDOLLE (1838) introduced the name *L. dregeana* for a South African species close to *L. virosa.*

FISCHER and MEYER (1846) described under the name *L. altaica* a form intermediate between *L. saligna* and *L. serriola* and originating from the Altai region. The leaves were described as entire, dentate or runcinate. The form from Kew has leaves which are too deeply lobed to be described as runcinate, but agrees otherwise in many respects with FISCHER's and MEYER's description. No herbarium specimens have been available for comparison and it is impossible to say whether the two forms are identical. If that is so, there is some reason for using the name *L. altaica* for the whole group, which is here referred to as primitive forms of *L. sativa.* THOMPSON, WHITAKER and KOSAR (1941) used, in their cytogenetic study, a form called *L. altaica* FISCH. and MEY. originating from Egypt, which probably also belongs to this group. Their illustrations show that it differs from the form from Kew in having non-lobed leaves. Whether it agrees with the form described by FISCHER and MEYER cannot be decided. From the above it is clear that there is no obvious choice of name for this group of forms.

Comparison with L. sativa and L. serriola. — There is considerable disparity between the different forms with regard to general morphology as shown by the following classification for presence of alleles for some of the most important characters.

Designation	Involucre	Anthocyanin	Spination	Leaf apex	Leaf form
Altaica	*b*	*CTr'i*	sp_1	*P*	U_{lob}
LC	*b*	*CTri*	*sp*	*P*	*u*
SL	*b*	*CtRI*	sp_2	*P*	*u*
SP	*b*	*CTRI*	sp_2	*P*	*u*
SB	*b*	*Ctrl*	*Sp*	*p*	*u*
SM	*b*	*CTRI*	*sp*	*P*	*u*
SN	*b*	*CTRI*	*sp*	*P*	*u*
ST	*b*	*Ct??*	sp_2	*p*	U_{lob}

The reader is referred to an earlier paper (LINDQVIST, 1960 b) for an account of the phenotypical manifestation of the various alleles and for a summary of their occurrence in *L. sativa* and *L. serriola.*

The new forms have all the allele *b* for erect involucre and thus agree with *L. sativa* with regard to this key character. Several of these forms have the allele *P* for pointed leaf apex and the alleles *R* or *r'* for the red or spotted type of anthocyanin pigmentation. These alleles occur in many forms of *L. sativa* but not at all in *L. serriola* or only as very rare exceptions, probably as a result of spontaneous hybridization with *L. sativa* (*cf.* page 326). Altaica and LC also have the recessive anthocyanin gene, *i*, which is common in *L. sativa* but not known in *L. serriola.* With regard to spination, all the four types described by LINDQVIST (1960 b) are found. Spination is often used as a character separating *L. serriola* and *L. sativa*, but it cannot be considered as a reliable key character since non-prickly forms occur also in *L. serriola* (*cf.* p. 324).

There is considerable variation with regard to shape of the panicle which in some forms, *e.g.* Altaica, approaches the flat-topped shape typical of *L. sativa* and in others, *e.g.* ST, is more pyramidal.

Presence of fine white hairs towards the apex of the achenes is one of the characters which distinguish *L. sativa* and *L. serriola* from the other species of the *serriola* group. Observations on the material used for this study have shown, that cultivated varieties are variable with regard to this character but they all have decidedly less hairy achenes than the *L. serriola* lines. Among the primitive forms, some agree with the most hairy of the cultivated forms, others agree with *L. serriola* and one, namely Altaica, has achenes which are even more hairy than those of *L. serriola.*

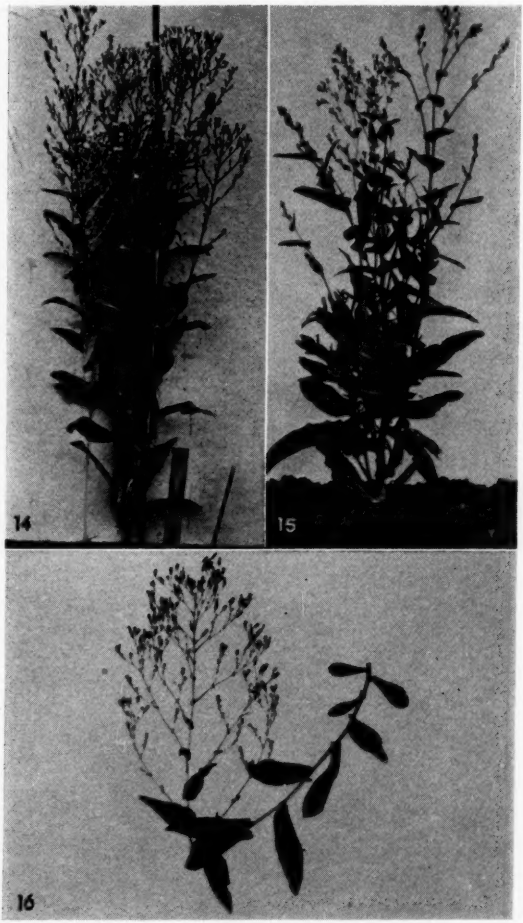
There are consistent differences between *L. sativa* and *L. serriola* with regard to some characters not mentioned earlier. Flower heads and achenes, or seeds as they will be called hereinafter for convenience, are decidedly larger in the cultivated species and the seeds germinate more rapidly and evenly when sown in the normal way. These statements are based on observations made on a large number of different lines over several years and the differences are exemplified by the data presented in Table 1. These data were obtained from plants grown in the same greenhouse and treated alike in every respect. The average diameter of 10 flower heads, with florets fully open, has been used as measure of flower head size, the length of the petals usually being closely related to the size of other floral parts. The line LC is exceptional in this

TABLE 1. *Flower head size and seed characters of L. serriola and L. sativa.*

Species	Line	Size of flower heads in mm	Average weight of seeds in mg	% germination in darkness 6-day count
L. serriola	Sc.L	14.3	0.78	5.5
„	Sc.H	10.6	0.62	16.4
„	Sc.R	13.1	0.73	8.9
„	Sc.Fr.	10.8	—	—
„	Sc.S.Afr.	11.3	0.58	8.0
„	Sc.J.	13.8	0.80	18.9
L. sativa				
1. cultivated	Berl.	20.1	1.34	72.2
„	R.rouge	17.8	1.13	95.8
„	Oakl.	19.5	1.28	47.8
„	Asp.	17.7	1.25	91.8
2. primitive	Altaica	15.1	1.10	97.6
„	SP	19.3	1.38	92.3
„	SL	17.7	1.37	95.3
„	SB	19.0	1.39	93.0
„	LC	12.7	1.48	86.4

respect, the petals being much shorter than usual in relation to other floral parts, and the figures for this line are therefore, in one sense, misleading. The germination tests were carried out in darkness at room temperature in petri dishes filled with sand. It is known that seeds of *L. serriola* are light-sensitive whereas most varieties of *L. sativa* germinate equally well in light and in darkness (HEGI, 1929). There are also differences with regard to temperature requirements, temperatures favouring the germination of one variety inhibiting the germination of another variety, and these differences can be eliminated if the seeds are exposed to low temperature during the initial stages of germination (BORTHWICK and ROBBINS, 1928; MC COLLUM, 1953). The conditions of the tests referred to here favoured the germination of cultivated varieties and retarded the germination of the *L. serriola* lines. The data in Table 1 show, that the primitive forms behaved in the same way as the cultivated forms of *L. sativa.*

On the strength of the above considerations, bearing in mind particularly that the new forms, like *L. sativa*, all have the erect involucre, it has been considered justified to include them under this name. Some of them are illustrated in Figs. 15—24. They all have a short period of

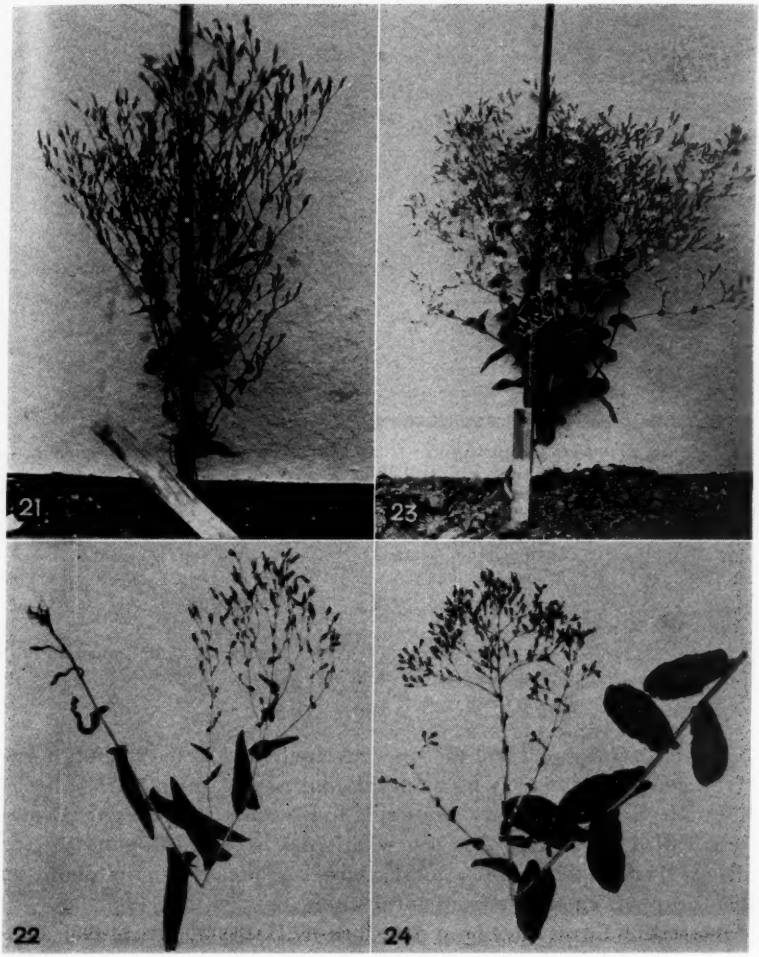


Figs. 14—16. *L. sativa*, cultivated specimens. — Fig. 14. Aspargus lettuce, line Asp.; growing plant. Fig. 15—16. The primitive form SL. Fig. 15. Growing plant. Fig. 16. Pressed specimen.

vegetative development, running to seed without a marked rosette stage. As plants for human consumption they appear to have very little to offer. In this connection it may be of interest to mention, that a similar form has recently been received from Aswan, Egypt, where it occurs in a state of semi-cultivation and is used as fodder for sheep, not for human consumption. This form is illustrated in Figs. 25 and 26. It is

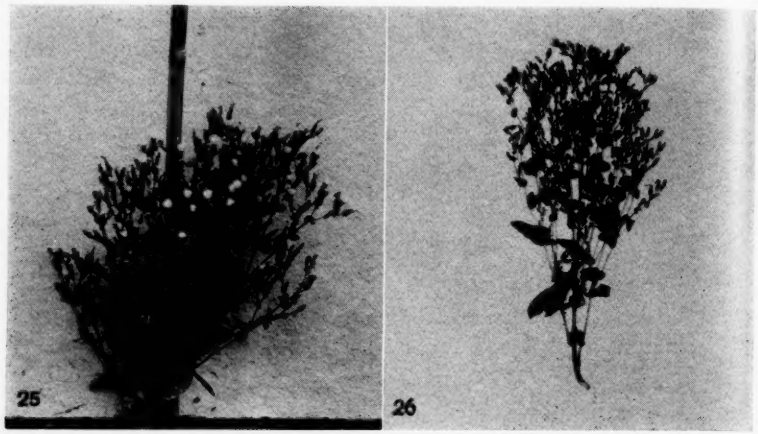
Figs. 17—20. *L. sativa*, primitive forms, cultivated specimens. — Figs. 17 and 18. Line LC. Fig. 19 and 20. Line Altaica. Figs. 17 and 19. Growing plants. Figs. 18 and 20. Pressed specimens.

possible that the other forms have been, and perhaps still are, used for similar purposes, but it has not been possible to obtain from the various botanic gardens any information about their initial origin.



Figs. 21—24. *L. sativa*, primitive forms, cultivated specimens. — Figs. 21 and 22. Line SM. Figs. 23 and 24. Line SB. Figs. 21 and 23. Growing plants. Figs. 22 and 24. Pressed specimens.

Similar forms in herbaria and literature. — A number of similar forms have been seen in various herbaria and are exemplified by the specimens listed below.

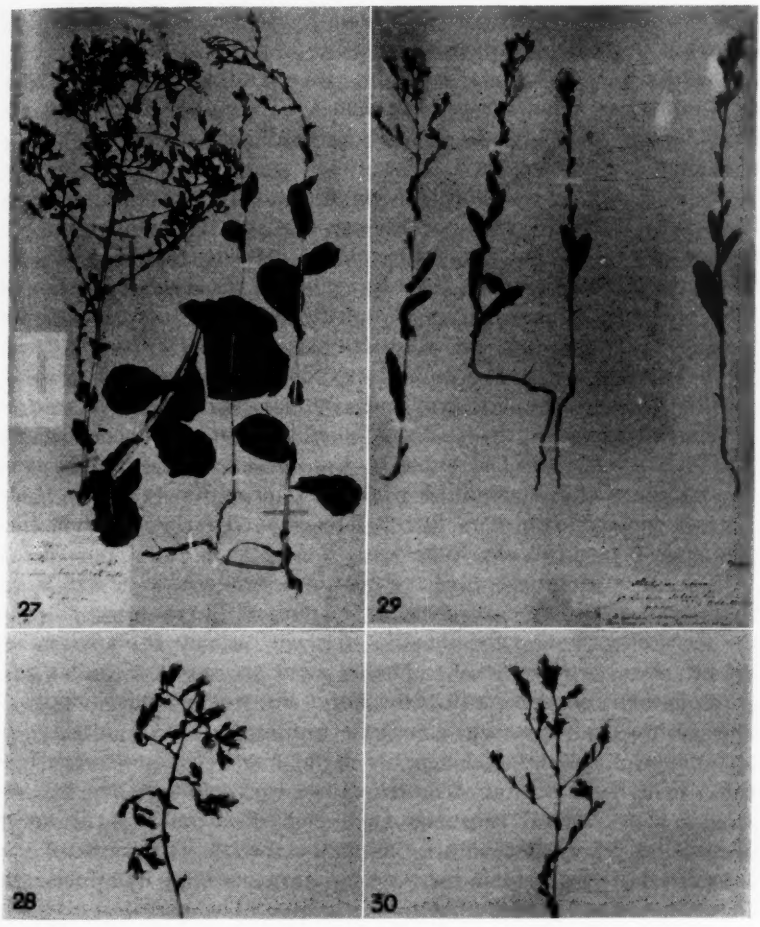


Figs. 25—26. *L. sativa*, primitive form from Aswan, Egypt, cultivated specimens. — Fig. 25. Growing plant. Fig. 26. Pressed specimen.

Herbarium	Name	Origin
Kew	*L. augustana*, ALLIONI	Aegypt sup.
„	*L. scariola*, *β sativa*	Egypt 1881. Escape?
„	*L. scariola*, L	Egypt 1867
Turin Herb Balbis	*L. Chaixii*	?
Lund	*L. altissima* M.B.	cultivated at St. Emiland, France
„	*L. cracoviensis* BUECK in herb.	„ „ „ „ „
„	*L. sagittata* W & K	„ „ „ „ „

These specimens all had the erect involucre and were certainly forms of *L. sativa*, but there is no evidence that any of them were collected from uncultivated habitats. According to BOISSIER (1875) a curly leaved form called *L. scariola β crispa* was found by HAUSSKNECHT in Kurdistan. HAUSSKNECHT (1895, p. 31), however, makes it quite plain that this form was an escape from cultivation.

Interesting forms have been described by HAUSSKNECHT (1895) under the name of *L. sativa* f. *spontanea* HAUSKN. and by ALEFELD (1866) under the name of *L. sativa* var. *Schulzii* ALEF. Specimens of the former from the HAUSSKNECHT herbarium at Jena are illustrated in Figs. 27 and 28 and specimens of the latter from Naturhistorisches Museum, Vienna, in Figs. 29 and 30. Both forms clearly have the erect involucre. *L. sativa* f. *spontanea* is strikingly similar to the line SB. The achenes are stated to be much more hairy than those of cultivated forms. HAUSSKNECHT's specimens of f. *spontanea* were cultivated by DIECK in an



Figs. 27—30. Herbarium specimens of primitive forms of *L. sativa*. — Figs. 27—28. *L. sativa* f. *spontanea* HAUSSKN. from Jena. Figs. 29 and 30. *L. sativa* var. *Schultzii* ALEF. from Vienna.

experimental garden in Zöschen, Germany. HAUSSKNECHT (1895) writes about the origin of this form '... einer Lactuca ..., deren Samen er (*i.e.* DIECK) aus den Bergen von Nerczynsk mit anderen Sämereien dort wild wachsender Pflanzen erhalten hatte'. On this evidence alone the possibility can, however, not be excluded that f. *spontanea*, like the

form received by the writer from Aswan, Egypt, was not a genuine wild form, but a primitive cultivated form.

ALEFELD (1866) was more definite concerning the wild form status of *L. sativa* var. *Schultzii*: 'Wild gewachsene Exemplare von dem Berge Arask Cool im westlichen Kordofan sammelte KOTSCHKY und bestimmte C. H. SCHULTZ BIPONTINUS . . .'

The following information is, however, found on the specimen (no. 765) from Kulturhistorisches Museum, Vienna: 'In montem Arasch Cool. Spontanea in cultis Sorghi frequens.' This, together with the fact that three similar specimens from Egypt were seen in the herbarium at Kew, one with the note Escape ?, and the fact that the form received from Aswan occurs in a semi-wild state, suggests that a form of *L. sativa* is found as a weed or in semi-cultivation in that part of the world. Another form growing in Egypt under similar conditions is *L. scariola* f. *oleifera* LAM., about which SICKENBERGER (1901) writes: 'spontanée dans la haute Egypte et la Nubie . . . Les indigènes laissent cette plant se propager et en recueillent les semances pour en retirer l'huile. Il n'y a pas de culture proprement dite'. Reference to oil extraction from *Lactuca* seeds is made also by KEIMNER (1924).

L. sativa apparently occurs in Egypt in a semi-wild state and is encouraged, rather than cultivated, for the purpose of extracting oil from the seeds or as fodder for animals. It is not difficult to imagine that the primitive forms referred to in this paper are used for similar purposes, perhaps in other parts of the world, but whether this is really so must for the present remain a matter of conjecture.

ALEFELD (1866) brought together in the Var — Gr — *silvestris* four varieties of *L. sativa:* var. *Schultzii* ALEF., var. *pallida* ALEF., var. *rufescens* ALEF. and var. *maculata* ALEF. and considered that this group of varieties represented wild forms of the species. The origin of var. *Schultzii* has already been discussed but ALEFELD gives no information about the origin of the other three varieties. The varieties *rufescens* with red leaves and *maculata* with spotted leaves are very interesting, because these pigmentation types are found also among the forms received by the writer from botanic gardens.

VAVILOV (1949—1950) states that *L. sativa* occurs as wild and cultivated in the Near Eastern centre of origin of cultivated plants, but no details are given about the wild forms referred to. As a result of literature studies HELM (1954, 1955) arrived at the conclusion that primitive forms of *L. sativa* once existed, which were characterized by long, entire, more or less pointed leaves and absence of a rosette stage. He

believed that such forms were common in LINNAEUS' days, and that they constituted his type for *L. sativa*. HELM (1954) calls these forms *L. sativa* provar. *sativa*, but thinks that they have gone out of cultivation and no longer exist.

There is reason to believe that the forms received by the writer from botanic gardens are similar to those described by ALEFELD, HAUSSKNECHT and HELM. It is possible that similar forms occur in a wild state, but there is no definite evidence on this point.

Are the primitive forms derivatives of crosses L. sativa×L. serriola? In view of the occasional occurrence of spontaneous hybridization between *L. sativa* and *L. serriola* the possibility must be considered that the presumed primitive types of *L. sativa* are products of such crosses. As an argument in favour of this assumption might be mentioned that they are in several respects intermediate between the two species and that they have been obtained from botanic gardens where opportunities exist for hybridization, and where hybrid products stand a much better chance of survival than in a natural habitat. There are, however, also a number of facts which argue against such an origin. It is difficult to understand why the alleged hybrid derivatives should consistently agree with *L. sativa* in the characters which separate the two species, *i.e.* the involucre character, size of flower heads and seeds and mode of germination of the seeds, unless these characters are strongly correlated, which according to preliminary investigations does not appear to be the case. If they were derivatives of *L. serriola×sativa* hybrids, some recombination of the characters would be expected. Nor is it easy to understand why they should all be characterized by very rapid development. Further, it is on general grounds more likely that these forms represent intermediate stages in the evolution of *L. sativa* than hybrids between the end result of this evolution and *L. serriola*. The latter alternative implies that all forms intermediate between cultivated lettuce and its ancestors have disappeared, which is difficult to accept, when forms exist, which have the expected characters. It is much more likely that the forms concerned really are primitive types of *L. sativa*.

II. ORIGIN OF CULTIVATED LETTUCE

1. History

The first records of lettuce as a vegetable come from Egypt. LAURENT TÄCKHOLM (1951) refers to tomb paintings from 4500 B.C. which show that a long leaved form of lettuce was in cultivation already then. KEIM-

NER (1924) classified the forms grown in ancient Egypt as belonging to *L. sativa* var. *longifolia*, *i.e.* the cos lettuce group, and points out that the illustrated specimens often have lanceolate, pointed leaves. From Egypt the cultivation of lettuce spread to Greece and Rome. Long leaved forms, presumably of the cos type, were used and probably also leaf lettuce types (HELM, 1954), but the cabbage lettuce did not appear until much later. The first incontestable record of the existence of cabbage lettuce comes according to HELM (1954, p. 124) from the 'Kräuterbuch' of L. FUCHS in 1543.

There is no certain evidence that the asparagus lettuce was used by the Egyptians, Greeks or Romans (HELM *l.c.*). These varieties are grown almost exclusively in China, where the lettuce was introduced between 600 and 900 A.D. (BREITSCHNEIDER, from HELM, 1954).

2. Previous theories

This subject has recently been reviewed by HELM (1954) and will therefore only be summarized briefly here.

The generally accepted theory is that cultivated lettuce originates from *L. serriola*. This opinion has been expressed among others by BISCHOFF (1851), DE CANDOLLE (1883) and more recently by DURST (1930), who considered that the differences and also the large variety of forms in the cultivated species are the result of accumulation of mutations due to selection during cultivation. This hypothesis has been accepted also by STEBBINS (1950, p. 292).

Different opinions have been expressed by ALEFELD (1866), who considered his var. *Schultzii* as the ancestor of *L. sativa* and by HAUSSKNECHT (1895) who believed that cultivated lettuce originates from *L. sativa* f. *spontanea* (see page 336). STURTEVANT (1886) believed that the three 'form-species', the lanceolate-leaved form (*i.e.* asparagus lettuce), cos lettuce and cabbage lettuce, originate from different wild forms which have been brought into cultivation in different regions. HELM (1954, p. 75) raises the question whether the cultivated forms have originated from pure *L. serriola* or from hybrids between this and other species, and cites as evidence for the latter alternative the marked morphological differences between cos lettuce varieties on one hand and leaf and cabbage lettuces on the other. According to FOCKE (1881, p. 221) LINNAEUS also considered the possibility that some of the cultivated lettuce varieties have hybrid origin.

Thus three different theories have been advanced concerning the

origin of cultivated lettuce, namely, 1: origin from wild forms of *L. sativa*, 2: origin through direct descent from *L. serriola* and 3: origin through hybridization between different species.

3. Discussion of different theories

Origin from wild forms of L. sativa. — With regard to this theory it has already been shown that there is no reliable evidence for the existence of genuine wild forms of *L. sativa.*

Origin through direct descent from L. serriola. — If it is understood by this theory that cultivated lettuce has originated from the species *L. serriola* as we know it to-day, two problems are involved, namely 1: why a species like *L. serriola*, which appears to have so little to offer as a crop plant, was taken into cultivation and 2: whether the polymorphism of *L. sativa* can be the result of mutations which have accumulated as the result of relaxation of natural selection.

With regard to the first question it is very interesting that *L. sativa* is known to occur in Egypt in a semi-wild state, being used, not for human consumption, but for extraction of oil from seeds or as fodder for animals. No information has been found about the properties of the *Lactuca* oil or the purpose for which it is used, and it is not known whether oil of similar quality is present in seeds of *L. serriola.* If this is so and if the wild species was used for the sake of this oil, there would be an obvious selective advantage for mutation from the dominant to the recessive allele at the *B*-locus, resulting in plants with the erect involucre and better seed retaining capacity. Non-prickly forms like ALLIONI's *L. augustana* might have found a use as fodder plants. Mutations towards a longer duration of the vegetative phase and more luxurious growth, might have led to forms similar to the long leaved Egyptian lettuce, f. *aegyptiaca*, and between this and the cos or asparagus lettuce the gap is not wide. However, the fact pointed out by KEIMNER (1924) that specimens of lettuce in ancient Egyptian tomb paintings invariably are illustrated with lanceolate, pointed leaves, does not very well fit into this picture, as it indicates that the character pointed leaf apex is a primitive trait in cultivated lettuce, although this character is hardly ever found in *L. serriola* as pointed out above. It may be argued that ancient paintings are not reliable evidence for the existence of morphological traits, but in this case authorities like KEIMNER and SCHWEINFURTH (quoted by KEIMNER *l.c.*) are in no doubt about the interpretation, and therefore the evidence cannot be ignored. SCHWEIN-

FURTH writes in a letter quoted by KEIMNER (*l.c.*): 'Man muss annehmen, dass in alter Zeit unter den Blattformen des angebauten Lattichs auch solche vorkamen, die durch gleichmässig spitz zulaufende Blätter ihre Abstammung von der Urpflanze *Lactuca scariola* L deutlich bekundeten. Auch in Europa haben wir in der als „Spargelsalat" bezeichneten Lattichform, in der *L. scariola* L. var. *angustana* ALL. eine der Stammform näherstehende Kulturvarietät mit spitz auslaufenden Blättern ...' Like many other authors (*cf.* p. 326) SCHWEINFURTH has apparently confused asparagus lettuce and ALLIONI's non-lobed form of *L. serriola*. However, his statement about the pointed leaf apex in the ancient Egyptian forms is unequivocal, and as this character is found also in asparagus lettuce and primitive forms of *L. sativa*, it is not unreasonable to assume that it has been a primitive trait in cultivated lettuce and it is therefore not likely that this originated from pure *L. serriola*, as we know the species to-day.

The most recently developed form of lettuce is the cabbage lettuce (*cf.* p. 340). It is not easy to imagine that this type has originated directly from cos lettuce although the difference between non-hearting and hearting habit is due to a single gene (LINDQVIST, 1960 b). The cabbage lettuces, like the leaf lettuces, have rounded leaves of a more tender texture and a different type of venation, which to STURTEVANT (1886) and HELM (1954) has suggested two different lines of descent within *L. sativa*. If that is so, the original forms must have been very closely related, as hybrids between cos and cabbage varieties are normal in every respect (THOMPSON, 1937; LINDQVIST, 1960 a). In this connection attention is drawn to the form received under the name *L. altaica*, which has been found to have only one of the dominant genes for rosette habit (LINDQVIST, 1960 b) and which has leaves more tender than those of cos lettuce varieties. Unfortunately nothing is known about the origin of *L. altaica*, but the possibility cannot be ruled out, that it occurs in a wild state in the Altai region, or perhaps in a semi-wild state like primitive *L. sativa* forms in Egypt, and that it may have played a part in the origin of cabbage lettuce.

Another possible mode of origin of cabbage lettuce must, however, be considered. Plants, which must be classified as cos lettuce, have repeatedly appeared by mutation in the cabbage lettuce variety Tezier (LINDQVIST, 1960 b) and somewhat similar rogues recurrently appear in the variety 456 (PEARSON, 1956). Whatever the mechanism involved, the reverse process is not inconceivable, and would result in the origin of cabbage lettuce from cos lettuce by means of a single change.

When the polymorphism of *L. sativa* is considered, doubts arise, however, as to whether this can be accounted for entirely by mutation. It is not difficult to imagine that characters which are controlled by genes recessive to those in *L. serriola* and which have a selective advantage in cultivated forms, owe their origin to mutation. Such 'Kulturpflanzenmerkmale' are erect involucre, lack of spination, bigger and more rapidly germinating seeds, bigger more tender leaves etc. (*cf.* SCHWANITZ, 1957; DARLINGTON, 1956). It is more difficult to accept such an origin for characters like pointed leaf apex and the spotted type of pigmentation, which have no obvious selective advantage in cultivation, which are controlled by dominant genes and which are lacking in *L. serriola*, but present in related species (*P* in *L. saligna* and *r'* in *L. virosa*). The presence of these characters in *L. sativa* lends some support to the theory that hybridization between different species has played a part in the origin of *L. sativa*, and this raises the question of the relative importance of mutation and hybridization as creators of polymorphism.

Gene mutation versus hybridization as cause of variability. — There is much controversy concerning the parts played by gene mutation and by hybridization in creating the variability necessary for evolution of cultivated plants from wild species. Some different points of view will be presented here.

LOTZY (1916) believed that hybridization is always the cause of variability in domestication, whereas SCHWANITZ (1957) was of the opinion that gene mutation alone, is sufficient to account for this variability and for evolution from wild to cultivated forms. HEISER (1949) concluded that mutation and hybridization both must have played an important part, but that very critical data are necessary to assess their relative importance.

Most of our crop plants are too old for their history to be unravelled, except by indirect means, but some of the ornamental plants are sufficiently young for their history to be accurately reconstructed. It must be assumed that the mode of evolution has been fundamentally the same for ornamental plants and for ancient crop plants. On evidence from the evolution of ornamental plants, DARLINGTON (1956, p. 165) concluded that cultivation by itself has never produced variability in a cultivated plant, but that new variation only arises as a consequence of a change in the breeding system, *e.g.* when cross-fertilizing species are forced to inbreeding, or when hybridization occurs between different races or species of self-fertilizing plants. He quotes three cases of ornamental plants in which a large number of very different varieties have

originated from a uniform wild species, namely *Lathyrus odoratus*, *Primula sinensis* and *Hyacinthus orientalis*, but in all three cases the ancestral wild type was a cross-fertilizer and bursts of variation occurred after some generations of inbreeding. DARLINGTON's explanation is that unstable recessive segregates have been selected which were prone to mutation.

Hybridization has often been emphasized as a very important factor behind the polymorphism of cultivated or wild plants. GRIGGS (1937) for example, points out that polymorphic domestic types are invariably the result of hybridization, whereas stable cultivated types have no close relations in domestication, with which they could be crossed. MÜNTZING (1930, p. 279) is of the opinion that interspecific hybridization is the main reason for the polymorphism of *Galeopsis tetrahit* and *bifida*. ANDERSON (1949) attaches much more importance to hybridization than to mutation for creating and maintaining variability, and believes that hybridization has been one of the most potent factors in the origin of domesticated plants and weeds. He proposed a very interesting theory concerning the origin of cultivated plants and associated weeds (ANDERSON *l.c.*). According to this theory primitive man unconsciously encouraged hybridization between wild species by removing barriers which had previously kept them apart, and by creating new habitats in which hybrid derivatives had a chance to survive. Out of mixtures of hybrids there originated, partly forms which were useful to man and therefore were taken into cultivation, and partly weeds which occupied waste place habitats round human habitations (so called 'camp followers'). Both were spread by man, and they became increasingly adapted and specialized, one by artificial, the other by natural selection. Thus the fact that a wild species is closely related to a cultivated species need not necessarily mean, that it is the ancestor of the cultivated species. Occasional hybridization with the cultivated species has, in combination wth the action of natural selection, the effect of increasing the efficiency of the weed species as a colonizing plant. The visible effect of such hybridization is drastically reduced because of a delicate balance between habitat and genotype and the cohesive effect of linkage between multiple factors on the morphological character complexes of the parental species, and as a consequence, the weed and the cultivated species remain distinct. The result is a slight trickle of genes from one species to another, by ANDERSON (*l.c.*) termed introgression, and this is in his opinion 'many times more important than mutation in keeping up the basic variability of the parental species'.

Possible role of hybridization in the history of L. sativa-serriola. — It has been proved that at least one species in the *serriola* group, namely *L. saligna*, is sufficiently closely related to the *L. sativa-serriola* ecospecies to give fertile hybrid derivatives, and some of these derivatives resemble lanceolate-leaved primitive forms of *L. sativa*. This makes it a real possibility that hybridization has played a part in the process of differentiation and in creating the polymorphism of *L. sativa*. More knowledge is desirable about related species from the Near East and Egypt, the areas which according to VAVILOV (1949—1950) form the centre of origin of cultivated lettuce. It is possible that in this area other forms or species exist, which are capable of hybridizing with the *L. sativa-serriola* ecospecies giving fertile derivatives.

There are certain facts which suggest that interspecific hybridization might have taken place during the evolution of *L. sativa*. It has already been pointed out that the presence of the characters spotted leaves and pointed leaf apex are easier to understand as a result of hybridization than of mutation, as the genes concerned are dominant and the characters found in related species.

The partial pollen sterility in some hybrids between different forms of the *L. sativa-serriola* complex may also be a consequence of hybridization. MÜNTZING (1938) and BERNSTRÖM (1953) suggest that the presence in *Galeopsis* and *Lamium* respectively, of partial intraspecific sterility not associated with meiotic disturbances, is due to minute structural chromosome differences, which owe their origin to hybridization with species differing in chromosome structure. A similar explanation is feasible for the partial sterility within *L. sativa-serriola*, although the possibility that it is due to gene mutations or spontaneous small structural changes cannot be ruled out on present evidence.

The disturbed segregations which have been observed with regard to leaf form (LINDQVIST, 1958, 1960 b) can also be interpreted as the result of interspecific hybridization. The cause of the abnormality was found to be different fertilization efficiency of the pollen grains, which presumably is due to complementary action of genes or small structural differences in the parents. As emphasized by MÜNTZING (1930, p. 316) there is no difference in principle between certation phenomena and partial pollen sterility. Similar abnormal segregations were found by BAENZIGER and GREENSHIELDS (1958) in crosses between *Melilotus alba* and derivatives of crosses between this species and *M. dentata* and *M. officinalis*. The authors are of the opinion that minute structural chromosome differences, resulting from the interspecific hybridization, are responsible for differences in efficacy of the gametes.

The difference between *L. sativa* and *L. serriola* with respect to the viability of their hybrids with other species may also be a consequence of hybridization. It is easier to imagine that the genes, or structural differences present in *L. sativa* and causing lethality in interspecific hybrids, have been introduced from another species, than that they should have originated spontaneously.

All the species of the *serriola* group are self-fertilizers and it has been shown earlier in this paper that evidence of spontaneous hybridization is rare. This is, however, based on observations in North West Europe and in U.S.A. It is well known that many plant species *e.g.* the tomato (RICK and BUTLER, 1956) are more or less allogamous near their centre of origin, but change to autogamy towards the periphery of their distribution area. It is therefore quite possible that the species of the *serriola* group are more allogamous in their distribution centre round the Mediterranean and hybridization between species more frequent which would make hybrid origin of *L. sativa* much more probable.

Any theory concerning the origin of *L. sativa* must take into account the very close relationship with *L. serriola*, and if the theory of direct descent is ruled out other explanations of the similarity between the two species must be considered. Three possibilities suggest themselves. 1: that both species have originated from heterogeneous hybrid populations, which included forms of some use to primtive man and that *L. sativa* has developed through selection by man, and *L. serriola* through adaptation to man-made waste habitats. 2: that the progenitors of *L. sativa* were hybrids between *L. serriola* and some other species. 3: that *L. serriola* is a product of hybridization between cultivated forms of *L. sativa* and some other species, in the same way as teosinte according to MANGELSDORF (1947) is a product of hybridization between cultivated maize and species of the genus *Tripsacum*, and not the progenitor of *Zea Mays*. On present evidence it is futile to speculate about which of these three possibilities is the most likely.

Evidence of occasional spontaneous hybridization between *L. sativa* and *L. serriola* has been produced in this paper. If such hybridization results in introgression of genes potentially useful to the wild species, hybrid derivatives with a selective advantage will occur from time to time and gradually oust other forms. In this way a close relationship between the weed and the cultivated species will be maintained. According to ANDERSON's theory, natural selection will reduce the visible effect of hybridization in the wild species and maintain the morphological differentiation. Introgression of genes from *L. sativa* has by AN-

DERSON been suggested as the reason for the ubiquity and aggressiveness of *L. serriola*. The related species *L. virosa* and *L. saligna*, which are separated from *L. sativa* by strong sterility barriers, certainly have a much more limited distribution.

Admittedly this discussion has been somewhat speculative, but in summing up the facts and arguments presented in this and in previous papers it may be said, with regard to the origin of cultivated lettuce, that many facts indicate that *L. serriola* is not the progenitor of cultivated lettuce, but that other species have been involved in evolution of this polymorphic form group, but it is not possible to form an exact picture of the events on the evidence available at present. The primitive forms must be considered as representatives of the ancestors of cultivated lettuce. The possibility that *L. sativa* and *L. serriola* are associated as cultivated species and 'camp following weed' is interesting and deserves further investigation.

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SUMMARY

An account of the taxonomy of the *serriola* group of *Lactuca* has been given, with special reference to the characters separating *L. serriola* and *L. sativa* and to primitive forms of the latter species. The nature of these forms and their bearing on the origin of cultivated lettuce has been discussed in the light of cytological and genetical results presented in earlier papers. A theory entailing hybridization between different species and evolution along the lines suggested by ANDERSON (1949) is preferred to the generally accepted theory of direct descent of *L. sativa* from *L. serriola*.

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SPECIES CROSSES WITHIN THE GENUS BRASSICA

II. ARTIFICIAL BRASSICA NAPUS L.

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I. INTRODUCTION

IN a previous paper (OLSSON, 1960), a report was given on the synthesis of *B. juncea* from crosses between *B. campestris* and *B. nigra*, and in the same paper the artificial material was compared with natural *juncea* in regard to different characters.

In a corresponding manner, *B. napus* L. can be synthesized by crossing *B. campestris* L. and *B. oleracea* L. By using different subspecies of the parents, it is possible to produce different subspecies of *B. napus*, too. Thus, at Svalöf, the two *napus* subspecies *oleifera* (METZG.) SINSK. (rape) and *rapifera* (METZG.) SINSK. (swede or rutabaga) were synthesized by using the *campestris* subspecies *oleifera* (METZG.) SINSK. (turnip rape) and *rapifera* (METZG.) SINSK. (turnip), respectively, in crosses with different subspecies and varieties of *B. oleracea*. In Japan, a promising fodder rape originated from crosses between an especially leafy form of *campestris* (ssp. *pekinensis*) and *oleracea* (HOSODA, 1950, 1953; MIZUSHIMA, in letter).

In the present paper, the artificial *B. napus* material will be compared with the mother species and with natural rape and swede. It is not possible, however, to make the comparison as complete as in the case of the *juncea* material mentioned above, mainly because of the considerable difficulties of wintering one of the parent species (*oleracea*) in the open at Svalöf. Since artificial swedes have already been the object of a preliminary report (OLSSON *et al.*, 1955), and since their practical value will be treated in greater detail elsewhere, the present paper will deal mainly with rape and only briefly with artificial swedes.

II. MATERIAL AND METHODS

The material of *B. oleracea* used in synthesizing *B. napus* included several varieties of green curled kale, marrow stem kale, dutch fodder kale, brussels sprouts, cabbage and kohlrabi. The material of *B. campestris* included different varieties of turnip rape and of turnip. Since the winter form of rape has a much greater practical value than the summer form, mainly winter forms of turnip rape were used in developing artificial rape. A survey of the varieties of the parent species forming the different artificial strains are given in Tables 3 and 4. Mainly the German variety Lembke's winter rape and Svalöf's Matador winter rape were used as standard material of natural rape, and the two varieties Svalöf's Fenix-Bangholm and Svalöf's Viktoria as standard material of swedes.

Crosses between the parent species were made both on the diploid and tetraploid level. Among the tetraploids, Erstling cabbage was obtained from dr. K. J. FRANDSEN, Øtoftegaard, Taastrup, Denmark, and Durabel cabbage from Mr. HYLTÉN-CAVALLIUS, Ohlsens Enke, Malmö. Other tetraploids used were developed at Svalöf (for method see OLSSON, 1960 p. 172).

In the report on artificial brown mustard, a brief description was also given of the development of autotetraploids of *B. campestris*, *B. nigra* and *B. juncea*. It was shown that autotetraploids of the species with low chromosome number were vegetatively well developed, whereas tetraploid *juncea* was considerably weaker than the corresponding diploid. The same general tendency is found in the present material. Tetraploid turnip, tested in a large number of trials, has given considerably higher yield than the corresponding diploid (JOSEFSSON, 1955, 1958). Tetraploid winter forms of turnip rape are, as the summer forms (*cf.* OLSSON, 1960), vegetatively well developed, but the seed yield of the tetraploids has been lower than that of the diploids. Even the tetraploid material of *B. oleracea* is vegetatively well developed. Tetraploid *napus* on the other hand shows the same weakness as tetraploid *juncea* (Table 1).

The methods for making the species crosses and determining cell size, fertility and chromosome number were the same as in the study of *B. juncea* (OLSSON, 1960). Some crosses were made in the greenhouse early in the spring, however, flowers being emasculated and pollinated as they developed, thus avoiding bag isolation. This procedure applied, for instance, in the spring of 1949, resulted in a remarkably high frequency of hybrids.

TABLE 1. *Comparison between diploid and tetraploid summer rape.*

Characters	Number of plants	Diploid	Tetraploid	Rel. val. 4x in % of 2x
Plant height, cm	50	117.4 ±1.83	89.4 ±2.05	76
Pollen fertility, per cent	50	95.7	88.5	91
Pollen diameter, μ	50	28.1 ±0.23	34.7 ±0.22	123
Number of good seeds per pod	50	22.2 ±0.72	6.1 ±0.37	27
Seed weight per plant, gram	50	6.5 ±0.66	1.5 ±0.22	23
1000-grain weight, gram	50	4.09±0.074	4.94±0.108	121

The artificial swedes obtained are denoted a.n.r. (*a*rtificial *n*apus *r*apifera) and, as in the *juncea* material, different plants are given running numbers. The material of artificial rape is denoted a.n.o. (*a*rtificial *n*apus *o*leifera), but one number may here refer to a single plant or a population, as, during the work with this material, all plants obtained in the same year from one cross combination are given the same number. In spite of the lack of uniformity in nomenclature it has been considered most convenient to retain this system here. The number of new amphidiploids included in one a.n.o. number can be seen in Table 3. For instance, a.n.o. 1 included only one plant, whereas all the 21 plants obtained in 1949 from the combination 4x Wilnensis turnip rape×4x kale are referred to as a.n.o. 4 and the individual plants as 4—1, 4—2 *etc.* If the same combination is repeated a second year, the resulting hybrids will be given a new main number. Different generations in the artificial material are denoted A_1, A_2, *etc.*, the index indicating the generation.

III. B. CAMPESTRIS L.×B. OLERACEA L. ARTIFICIAL B. NAPUS L.

1. Synthesis

From artificial crosses between diploids of *B. campestris* and *B. oleracea*, Japanese investigators already in 1930—32 succeeded in producing artificial rape (U, 1935). In their crosses, U and his collaborators obtained 4 hybrid plants, denoted COF_1-I, -II, -III and -IV, with 19, 28, 29 and 38 chromosomes, respectively. The first of these had one genome (a) from *campestris* and one (c) from *oleracea*, while the three others had the constitutions acc, aac and aacc. The last-mentioned plant, thus, had the genome constitution of *B. napus*, and, in addition, agreed with this species in morphology and fertility. Unreduced ga-

metes functioned in the 19 chromosome hybrid yielding 38 chromosome F_1 plants.

Later on, artificial *napus* has been synthesized from crosses between *campestris* and *oleracea* among others by FRANDSEN (1947), RUDORF (1950), MIZUSHIMA (in letter), HOSODA (1950), KOCK and PETERS (1953), OLSSON (1953), OLSSON *et al.* (1955) and HOFFMANN and PETERS (1958), the last-mentioned authors reporting in detail on the different cross combinations which may be used for a direct or for a more indirect synthesis of *B. napus*.

Table 2 gives a summary of crosses made at Svalöf in 1948—1957 with the aim of synthesizing artificial *napus*. By crossing diploid forms of *campestris* with *oleracea* a total of 16 hybrid plants were obtained, while the reciprocal combination gave no result. That the cross may be possible even in the latter direction was shown among others by HOFFMANN and PETERS (1958).

In some instances diploids and tetraploids were crossed with each other, the combination tetraploid *campestris*×diploid *oleracea* resulting in three hybrid plants. The main part of the crosses were, however, performed between tetraploids of the parent species. In this case, hybrids were obtained with *campestris*, as well as with *oleracea*, as mother. Of the 133 hybrid plants obtained from tetraploid *campestris* ♀×tetraploid *oleracea* ♂, 103 were obtained from crosses made in greenhouses in the spring of 1949, all of them being derived from 4 mother plants. The remaining 30 hybrids were produced from several mother plants. Among the 130 hybrids of the reciprocal combination no less than 126 originated from one single mother plant, this cross also having been carried out in the greenhouse. The remaining 4 hybrid plants derive from 4 different cross combinations.

In agreement with earlier experiences of BECKER (1952) and HOFFMANN and PETERS (1958) it was found that seed setting was better in a cool greenhouse than in a warmer one or in the field. HOFFMANN and PETERS were not able to demonstrate any differences in crossability between individual plants. The present author, however, is of the opinion that the genotype and physiology of the parent plants have an important influence upon the results of the crosses which is in agreement with the ideas of MIZUSHIMA (1952). The great differences in number of hybrids obtained by different authors (RUDORF, 1950; HOFFMANN and PETERS, 1958) when crossing diploid *campestris* and *oleracea* may, at least partly, be explained by individual differences between parent plants.

TABLE 2. *Results of crosses between B. campestris and B. oleracea.*

	Cross combination	Chromosome number	Year	Number of pollinated flowers	Number of harvested seeds	Number of F_1-plants
Crosses including turnip rape	*B. campestris*×*B. oleracea*	2x×2x	1949—1957	7434	3994	15
		4x×4x	1948—1957	13867	2246	129
	B. oleracea×*B. campestris*	2x×2x	1949—1956	2456	246	0
		4x×4x	1948—1957	12190	1048	130
			Total	35947	7534	274
Crosses including turnip	*B. campestris*×*B. oleracea*	2x×2x	1952—1955	2961	793	1
		2x×4x	1954—1955	1317	1237	0
		4x×2x	1952—1955	1271	108	3
		4x×4x	1952—1955	9017	1105	4
	B. oleracea×*B. campestris*	2x×2x	1952—1955	1618	100	0
		2x×4x	1953—1955	2331	121	0
		4x×2x	1954—1955	1382	63	0
		4x×4x	1952—1955	6684	387	0
			Total	26581	3914	8

As seen from Table 2, crosses between tetraploid parents gave considerably more hybrids than corresponding crosses between diploids, a result contrary to that obtained by HOFFMANN and PETERS (1958). Probably, the constitution of individual parent plants rather than their ploidy level may be responsible for such differences, as well as for the success of crosses in generally. A similar phenomenon may underly the fact that turnip, when used as a parental component, gave a significantly lower seed setting than turnip rape. The difficulty in choosing parents for the production of artificial rape is further illustrated by the fact, that while FRANDSEN (1947) in crossing tetraploids of *campestris* and *oleracea* obtained 65 hybrid plants from 3000 pollinated flowers, HOFFMANN and PETERS (1958) obtained no single hybrid plant from more than 9000 pollinated flowers.

Since *B. oleracea* is partly self-sterile, single plants of this species were placed in greenhouse chambers or in field isolations of *B. campestris* with the hope that the rich supply of *campestris* pollen should result in spontaneous hybrids between *oleracea* and *campestris*. Most of the seed harvested from the *oleracea* plants was the result of selfing, but in all 8 rape-like plants were obtained. Probably these plants actu-

TABLE 3. *Survey of artificial rape and trigenomic hybrids obtained from crosses between B. campestris ssp. oleifera and B. oleracea and some data of A_1- and F_1-plants.*

A. n. o. number	Cross combination	Year—number	Number of A_1 and F_1 plants	2n	Pollen-fertility %	Seed fertility %	Number of good seeds per pod
1	4x Erstling cabbage×4x Wilnensis turnip rape	1949—101—23—1	1	38	97	97	21.8
2	4x Lembke's turnip rape×4x Durabel cabbage	1949—102—1—1	1	38	99	82	15.5
3	4x Erstling cabbage×4x Lembke's turnip rape	1949—102—12—b	1	38	99	92	21.1
4	4x Wilnensis turnip rape×4x Low green curled kale	1950—101	21	38—41	73	18	4.5
5	4x Lembke's turnip rape×4x Low green curled kale	1950—102	82	38—41	80	19	4.4
6	4x Lembke's turnip rape×4x Durabel cabbage	1950—103—2	1	37±	83	10	2.3
7	4x (Marrow stem kale×Green kale)×4x Wilnensis turnip rape	1950—105—3	1	38	98	71	19.7
8	4x Wilnensis turnip rape×4x "Fest und Viel" brussels sprouts	1951—101	2	—	71	13	3.2
9	4x Lembke's turnip rape×4x "Fest und Viel" brussels sprouts	1951—102	5	—	83	8	2.2
10	4x Wilnensis turnip rape×4x "Fest und Viel" brussels sprouts	1952—223	4	38—40	75	16	5.3
"11"	2x Lembke's turnip rape×2x Marrow stem kale	1952—226	7	29	64	6	2.1
12	4x „ „ „ ×4x „ „ „	1952—227	4	38	71	18	6.3
"13"	2x „ „ „ ×2x Sutton's New Year savoy cabbage	1952—226	6	29	66	7	2.4
14—15	4x „ „ „ ×4x „ „ „ „ „	1952—227	6	38—39	72	12	4.0
16	4x „ „ „ ×4x "Fest und Viel" brussels sprouts	1952—227—23	1	38	72	—	—
17	4x Marrow stem kale×4x Lembke's turnip rape	1952—228—7	1	38	97	72	21.5
"18"	2x Strube's summer turnip rape×2x Marrow stem kale	1953—369—17—1	1	29	48	7	1.8
19	2x Rapido turnip rape×2x Dutch fodder kale	1954—703—1	1	19—38	94	95	27.3
22	4x Wilnensis turnip rape×4x Low green curled kale	1955—319—1	1	38	98	88	23.5
26	4x New Kastrupsgaard brussels sprouts×4x Wilnensis turnip rape	1957—320	126	—	90	—	—
27	4x Gruber's turnip rape×4x Red garnishing kale	1958—1622	1	—	55	22	5.0

Lembke's, Wilnensis, Rapido and Gruber's turnip rape are winter forms; Strube's is a summer form.

ally represented artificial rape, but since they were not distinctly different from natural rape it could not be definitely proven that they were not intermixtures. Hence, they will not be further dealt with here. This method of producing artificial rape should, however, be further tested.

Hybrid seeds obtained from artificial crosses were often smaller than the simultaneously obtained matromorphous seeds. In many cases, however, the F_1 plants derive from seeds of normal size. According to HÅKANSSON (1956), many hybrid embryos abort because of faulty endosperm development and lack of nutrition of the embryo. Thanks to a suitable technique for embryo culture, NISHI, KAWATA and TODA (1959) obtained a much higher frequency of hybrids than was possible when the embryos were left to develop on the mother plant.

In order to simplify the further treatment of the material, Tables 3 and 4 present a summary of the artificial rape and artificial swedes produced at Svalöf from artificial crosses between *campestris* and *oleracea*.

2. Chromosome numbers and morphology of the hybrid plants obtained

Of the 16 hybrids obtained from crosses between diploid forms of *campestris* and *oleracea* only one single plant — a.n.r. 9 (Table 4) — had 2n=19. It was completely sterile but was kept alive, from 1955 to 1959, by vegetative propagation. Some of the cuttings were treated with colchicine and yielded 38 chomosome progeny.

One plant — a.n.o. 19 — was fully fertile and self-compatible with 95 per cent well developed seed and 27.2 good seeds per pod. The generative tissue of this plant had 38 chromosomes, but three of the root tips studied had 19 chromosomes and three 38 chromosomes. Obviously, the plant was a chimaera, even if the 19 chromosome sector was small and never resulted in flowering branches. A.n.o. 19 must have become amphidiploid as a result of a somatic chromosome doubling, and this doubling must have occurred early in the ontogeny, although after the first division of the zygote. Because of its origin a.n.o. 19 is completely homozygous.

The remaining 14 hybrids, from crosses between diploid parents, all had 2n=29. The meiosis showed $10_{II}+9_{I}$ and the plants thus contained two *campestris* and one *oleracea* genomes (aac). They must have originated from reduced male gametes and unreduced or otherwise doubled egg cells. These plants were comparable, in their chromosomal constitu-

TABLE 4. *Survey of hybrids obtained from crosses between B. campestris ssp. rapifera and B. oleracea and some data of the A_1-plants.*

A. n. r. number	Cross combination	Year—number	2n	Pollen fertility %	Number of good seeds per pod
1	4x Yellow Tankard turnip × 2x Low green curled kale	1953—118—1	38	90	
2	ditto	1953—118—2	38	97	
3	4x Östersundom turnip × 2x "Fest und Viel" brussels sprouts	1953—123—1	38	98	
4	4x Yellow Tankard turnip × 4x (Marrow stem kale × green kale)	1953—4—1	—	69	
5	ditto	1953—4—2	—	70	
6	4x Silage turnip × 4x "Wiener weiss" kohlrabi	1954—354—1	38	97	0.7 [1] / 20.5 [2]
7	4x Vege turnip × 4x "Wiener blau" kohlrabi	1954—495—1	38	90	22.8
9	2x (Bortfelder × Dales hybrid) × 2x "Wiener weiss" kohlrabi	1955—1015—1	19 38	0 83	

[1] Self-pollinated; [2] Cross-pollinated.

tion, to hybrids between rape and turnip rape, but in spite of this they were in most cases morphologically much more similar to rape than such hybrids usually are, in this respect corresponding with U:s COF_1-III. These F_1 hybrids had a strongly reduced fertility: 63.5 per cent good pollen, 6.3 per cent well developed seeds and 2.2 good seeds per pod. The F_2 material was very heterogenous but included fully fertile plants. It was impossible to decide whether these plants descended from crosses between different 29 chromosome F_1 plants or from such plants crossed with natural rape. The 29 chromosome F_1:s were grown in isolation greenhouses, separated from other material, but since the greenhouses were not quite pollen-proof, spontaneous crosses cannot be completely excluded. The fertile F_2 plants gave rise to the artificial — or possibly semiartificial — rape strains denoted a.n.o. 11, 13 and 18.

The three plants of artificial swedes (a.n.r. 1—3), derived from crosses between tetraploid turnip and diploid kale or brussels sprouts, all had 2n=38 and were fully fertile. Evidently they were formed by the functioning of unreduced pollen.

Among the artificial material of *napus* from crosses between tetra-

TABLE 5. *The relation between the lengths of the broad and the narrow parts of the petals and between the lengths of the long and the short stamens in artificial and natural rape, kale and turnip rape.*

Year	Material	Number of plants	Petals broad parts/narrow parts $\bar{x} \pm m$	Stamens long/short $\bar{x} \pm m$
1950	Tetraploid green curled kale	25	1.22±0.014	1.12±0.0076
	Natural rape, Lembke's	25	1.73±0.017	1.29±0.015
	A.n.o. 4_{1-21}, A_1	21	1.73±0.033	1.44±0.019
	A.n.o. 5_{1-50}, A_1	50	1.86±0.018	1.50±0.018
	Tetraploid Wilnensis turnip rape	25	1.75±0.038	1.58±0.028
	Tetraploid Lembke's turnip rape	25	1.96±0.053	1.57±0.032
1952	Diploid green curled kale	25	1.45±0.027	1.16±0.018
	Natural rape, Lembke's	25	1.84±0.022	1.30±0.016
	A.n.o. 1, A_2	25	1.82±0.025	1.43±0.026
	„ 3, „	25	1.82±0.019	1.39±0.033
	„ 6, „	25	2.01±0.036	1.39±0.021
	„ 7, „	25	1.83±0.031	1.37±0.011
	Diploid Wilnensis turnip rape	25	2.04±0.040	1.72±0.045
	Diploid Lembke's turnip rape	25	2.10±0.037	1.51±0.033

ploids of *campestris* and *oleracea*, a.n.o. 1, 2, 3, 7, 12, 16, 17 and 22 and a.n.r. 6 and 7 had 2n=38, in a.n.o. 4, 5, 10 and 14, plants were found with 2n=38, as well as with one or more supernumerary chromosomes. The chromosome numbers of the A_1 plants of a.n.o. 8, 9, 26 and 27 and a.n.r. 4 and 5 were not determined, but judging from the morphology of the A_1 plants, and the chromosome numbers of the A_2 plants, there is reason to assume that a.n.o. 8, 9 and 27 and a.n.r. 4 and 5 had aneuploid numbers round 38 while a.n.o. 26 had 2n=38. In many cases it was difficult, or even impossible, to determine exactly the chromosome number of a plant.

The artificial material of rape as well as of swedes is morphologically intermediate between the parent species and more or less resembling the corresponding natural forms. For comparative morphological studies, certain measurements were performed on the primary species and on natural and artificial rape.

Table 5 shows the relations between the lengths of the broad and the narrow parts of the petals and between long and short stamens. The measurements were made with the same methods as in *B. juncea* (OLSSON, 1960), three petals or stamens per plant being measured. As seen,

TABLE 6. *Length in mm. of pod valve and beak in artificial and natural B. napus.*

Variety	Number of plants	Valve length $\bar{x} \pm m$	Beak length $\bar{x} \pm m$
Natural rape, Lembke's	50	66.8±0.84	11.9±0.25
A.n.o. 1	48	65.0±0.99	12.2±0.38
Sv 55/1069 from a.n.o. 5	50	63.7±1.03	11.3±0.35
A.n.o. 7	49	58.5±0.73	12.7±0.26
Sv 55/1073 from a.n.o. 13	49	64.8±1.63	12.0±0.25

the differences are smaller between *oleracea* and *campestris* than between *campestris* and *nigra*; in some cases no significant differences in the relation between the two parts of the petals were found between natural or artificial rape and tetraploid turnip rape. Table 6 gives some results of the pod measurements in natural and artificial rape.

It is not possible to predict the characters of the hybrid from those of the parents, and the possibility to synthesize hybrids of a desired type by a choice of parents is rather limited. It is, however, possible to trace some characters in the hybrids back to the parents. As an example, swedes were obtained only when turnip was used as one of the parents. On the other hand, the use of turnip does not warrant the hybrid to become a swede, since types with a typical rape root may also appear (HOFFMANN and PETERS, 1958; "a.n.r." 6 in the present study). Curly leafed types of artificial rape were obtained from combinations were the *oleracea* parent was cabbage (a.n.o. 1) or marrow stem kale (a.n.o. 17), while combinations with green curled kale as parent did not show any marked curliness of the leaves. On the other hand, the rounded leaf shape of a.n.o. 26 has a clear resemblance to brussels sprouts. Types which are markedly low growing in the autumn have been obtained, among others in a.n.o. 11 from Lembke's turnip rape×marrow stem kale, although the latter has a decidedly tall stem.

In order to give an idea of the similarity between natural and artificial rape and swedes, some of the artificial types will be described more in detail.

A.n.o. 1 from 4x Erstling cabbage×4x Wilnensis winter turnip rape consisted of one A_1 plant with 2n=38. Morphologically the A_1 plant was almost identical to rape, with pronouncedly waxy, broad rosette leaves with only very few hairs. In late autumn the inner rosette leaves, and in spring the lower and middle stem leaves, were strongly curled (Fig. 2), a.n.o. 1 being distinctly different in this respect from any natural rape studied at Svalöf. Selection in later generations resulted in curly leaved

Fig. 1. Lembke's rape and a.n.o. 1, 2 and 3 in the rosette stage.

as well as smooth leaved families. The flower colour was lighter than that of Lembke's rape but deeper than, *e.g.*, light-coloured plants of Mansholt's rape. In later generations there occur different light yellow variants. The pods were horizontal, long and narrow with thin walls. There were often 1—2 seeds in the beak. The A_1 plant was fully fertile and self-compatible and gave, when selfed in the greenhouse, 21.8 seeds per pod. In flowering and ripening a.n.o. 1 is 2—3 days later than Lembke's rape. The winter hardiness is somewhat lower than in this variety, and the yield about 15 per cent inferior.

A.n.o. 2 from 4x Lembke's turnip rape $\times$4x Durabel cabbage consisted of one A_1 plant with 2n=38. The rosette leaves of this plant were characterized by a long, narrow end lobe and only 1—2 side lobes (Figs. 1—2). The leaves were strongly waxy with only very few hairs. The petioles of the leaves at the middle of the stem had a narrow wing border, broadening at the base and partly clasping. The flower colour was lemon yellow. The pod peduncle was long standing at an angle of 70—80° to the stem. The pods were comparatively short with a marked beak without seeds. The A_1 plant was fully fertile and self-compatible but had fewer seeds per pod than *e.g.* a.n.o. 1 and a.n.o. 3. Sown in the autumn a.n.o. 2 shows a distinct tendency to shoot and has a very low overwintering capacity; sown in the spring it behaves like a sum-

Fig. 2. A.n.o. 1, 2 and 3 in the bud stage.

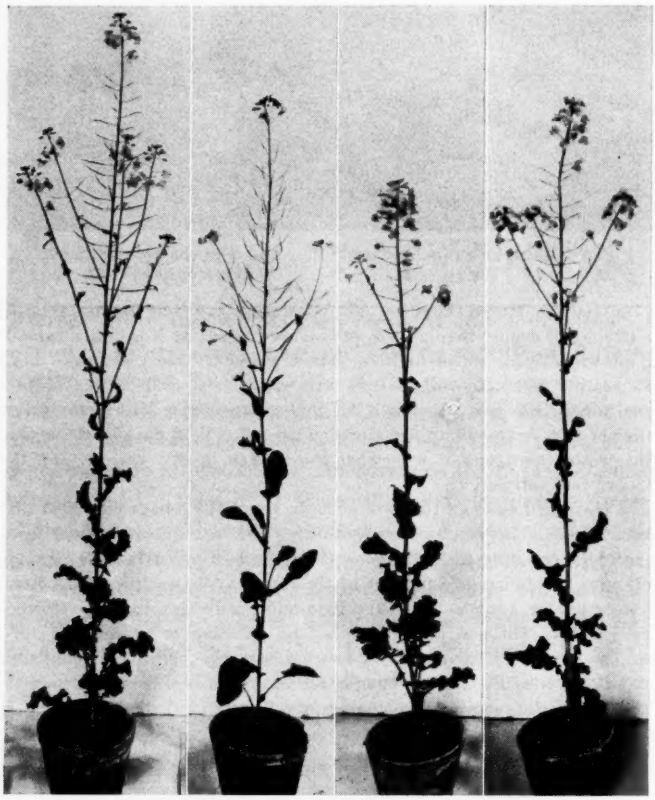


Fig. 3. A.n.o. 1, 2 and 3 and Lembke's rape, flowering plants.

mer form. The stem is very weak. The characteristic leaf shape, the short pods and the weak stem constitute strong evidence that a.n.o. 2 is a new synthesis.

A.n.o. 4 from 4x Wilnensis winter turnip rape×4x low green kale. 21 A_1 plants with 2n=38—41. There was evident variation between different A_1 plants, but all of them were more or less intermediate between rape and turnip rape. The rosette leaves were more lobed and more hairy than in typical rape, but less so, than in turnip rape. The petiole and, to a smaller extent, the lamina had a certain waxiness, but this was less marked than in rape. The stem leaves showed evident similarity to turnip rape, but the upper ones were less clasping than in this species. The buds of a.n.o. 4 were definitely longer, narrower and more waxy than those of tetraploid turnip rape. The flowers and pods of A_1 had several characteristics of turnip rape, such as a short "pedicel" part of the petal and a fairly long beak on the pods. The flowers were lemon yellow, no segregation in flower colour occurring.

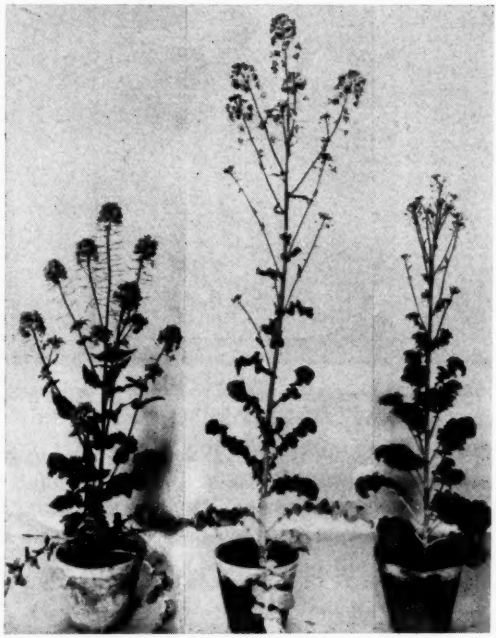


Fig. 4. In the middle, an A_1-plant of a.n.o. 5 from a cross between tetraploid *B. campestris* (to the left) and tetraploid *B. oleracea* (to the right).

Although A_1 of a.n.o. 4 was grown in a greenhouse under ideal conditions and with a plentiful supply of bees the fertility was low with an average pollen fertility of 73.3 per cent and 4.5 seeds per pod. The fertility in later generations is seen from Table 8. Most of the A_1 plants were self sterile. The A_2 material was highly variable, and in later generations it was possible to select fully typical rape plants.

A.n.r. 1 and 2 were obtained from the cross 4x Yellow Tankard×2x green kale, but had 2n=38 and were fully fertile and self-compatible. The swelling of the root is marked, the shoulder of the root is reddish violet. In regard to root development, leaf colour, flower colour and pod shape, a.n.r. 1 and 2 agree very well with swedes of the Bangholm type. The progeny, especially of a.n.r. 1, shows a considerably wider variation in root shape, however, than does natural swede (Fig. 10).

A.n.r. 3 and 7 are very similar although derived from different crosses (Table 4). They are fertile and self-compatible. The root is swollen, and of a greenish yellow colour. A.n.r. 3 is very uniform and rather similar to the Swedish variety Viktoria in leaf as well as in root shape. The roots of a.n.r. 7 are smaller.

A.n.r. 4 and 5 from a cross 4x Yellow Tankard×4x (marrow stem kale×green kale) are morphologically more or less intermediate between turnip and swede. At the same time they have a low fertility and on the whole behave just as the already described a.n.o. 4.

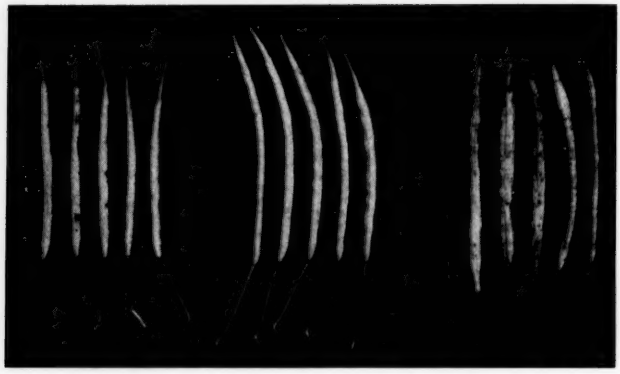


Fig. 5. Pods from a.n.o. 5 (A_8) in the middle and from *B. campestris* (to the left) and *B. oleracea* (to the right).

"A.n.r." 6 from 4x silage turnip×4x kohlrabi has 2n=38, is fully fertile but at the same time strongly self-incompatible (Fig. 8). It is no doubt a *napus* but is very different from the swede. The flowers are lemon yellow as in rape, the root is thin and woody instead of swollen and fleshy. When sown in the autumn it behaves as a winter rape.

A.n.r. 9 was obtained from 2x (Bortfelder×Dale's hybrid)×2x kohlrabi. The primary A_1 plant was almost identical to *napus* but had 2n=19 and was completely sterile. Colchicine treatment of cuttings resulted in 38 chromosome, fertile, plants, which gave seed upon free flowering and after spontaneous crossing with swedes. The plants from these seeds had swollen roots.

The observations on the morphology of the material of artificial *B. napus* here studied may be summarized as follows: There are several types which cannot be morphologically distinguished from natural *napus*. In other cases, the new material agrees in all essential characters with natural *napus*, but differs so distinctly in a few details from all available material of natural *napus*, that it may be very safely concluded that new syntheses have occurred. Finally, some materials are more or less intermediate between rape and turnip rape in several characters, such as leaf colour, hairiness, length of beak etc. In some cases this is caused by the presence of supernumerary *campestris* chromosomes, but this is presumably not always the reason for the intermediate morphology. In such material, a few years of selection towards an increase in fertility will influence also the morphology, so that the material becomes completely rape-like.

TABLE 7. *Pollen diameter in μ in artificial and natural B. napus. Belling glycerine slides.*

Year	Variety	Number of plants	Per cent good pollen	Pollen diameter in μ $\bar{x} \pm m$
1950	A.n.o. 4_{1-21}, A_1	21	73.3	28.7 ± 0.22
	A.n.o. 5_{1-82}, A_1	82	80.2	28.3 ± 0.11
	Natural rape, Lembke's	50	97.4	28.9 ± 0.18
1951	A.n.o. 7, A_2	50	96.7	29.5 ± 0.18
	Natural rape, Lembke's	50	97.0	29.0 ± 0.17

3. Cell size

When measuring the cell size of artificial *juncea*, it was found that stomatal cells and pollen grains of the A_1 material as a rule were larger than the corresponding cells in the natural material, and first after a few years of cultivation the artificial material attained the same cell size as natural *juncea* (OLSSON, 1960). A couple of A_1 plants of artificial *juncea* (a.j. 23 and 32), however, had the normal cell size of the species.

In the material of artificial *B. napus* measurements of cell size have not been made to the same extent as in the *juncea* material. Especially, it has not been feasible to follow possible changes in cell size through the generations. Certain data exist, however, indicating an increased cell size in newly synthesized rape. Thus, FRANDSEN (1947) states that there is an increase in pollen size, but no difference in the size of stomatal cells, between natural and artificial *B. napus*. In determining the pollen size in artificial rape, RUDORF (1950) found good agreement with natural material, except for one strain which had considerably larger pollen grains. Even in the present material, certain differences in pollen size were observed between different artificial materials. Since, however, some of the artificial strains had somewhat larger pollen grains than Lembke's rape and others had smaller pollen than the natural variety, no general tendency towards an increase in cell size was found in the artificial material (Table 7).

4. Fertility

The fertility of the A_1 plants is listed in Tables 3 and 4. As seen from these tables several A_1 plants, both of artificial rape (a.n.o. 1—3, 7, 17,

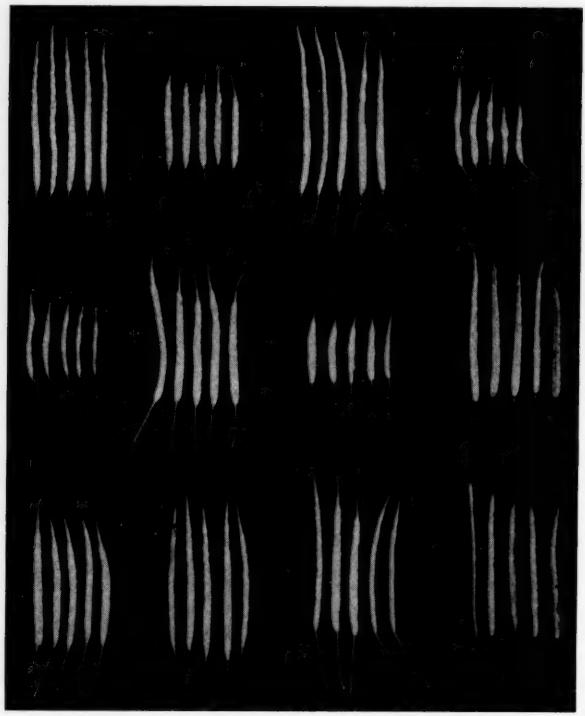


Fig. 6. Pods from different A_1-plants of artificial rape and from natural rape. — In the upper row a.n.o. 1, 2, 3 and 4. In the middle row a.n.o. 5, 7, 9 and 19. In the lower row natural rape.

19, 22) and of artificial swede (a.n.r. 1—3 and 6—7) were fully fertile. In other A_1:s — such as a.n.o. 4—6, 8—10, 12, 14—16 and 22 and a.n.r. 4—5 — both pollen and seed fertility were considerably lower than in the natural species (Fig. 6). If the fertility of the present A_1 material of *B. napus* is compared with the corresponding material of *B. juncea*, it is found that the *napus* material includes a greater number of fully fertile A_1:s than the *juncea* material, and that at the same time the reduction in fertility in the rest of the *napus* material has been much less severe than the corresponding reduction in *juncea*. The pollen fertility of A_1 plants of a.j. 1—22 was about 30 per cent (OLSSON, 1960), while in partially fertile A_1 plants of *napus* it was 70—80 per cent.

A few generations of continuous selection for increased number of

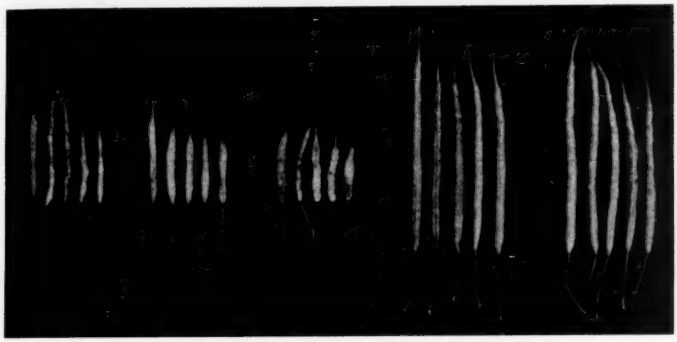


Fig. 7. The effect of selection for number of seeds per pod in a.n.o. 5. To the left pods from three A_2-plants; to the right from two A_5-plants.

good seeds per pod in the *napus* material resulted, just as in the *juncea* material, in a marked increase both in pollen fertility and in number of seeds per pod (Fig. 7). In order to illustrate this trend, Table 8 gives pollen fertility and number of seeds per pod in A_2 to A_5 material of a.n.o. 4 and 5 from a trial in 1955 where all generations were tested side by side. In the population a.n.o. 4, the pollen fertility improved from 81 per cent in A_2 to 98 per cent in A_5 and at the same time the number of good seeds per pod increased from 3.3 to 16.7. In the same way, the population of a.n.o. 5 showed an increase in pollen fertility from 87 to 96 per cent and in number of good seeds per pod from 3.9 to 16.9.

TABLE 8. *The effect of selection for number of seeds per pod in artificial rape 4 and 5. The different generations tested in 1955.*

Variety	Generation	Pollen fertility in %		Number of seeds per pod	
		n	$\bar{x}$	n	$\bar{x} \pm m$
Mass-selection in a.n.o. 4	A_2	30	80.5	34	3.3±0.35
,, ,, ,,	A_3	30	83.5	30	4.1±0.36
,, ,, ,,	A_4	49	95.4	45	11.9±0.94
,, ,, ,,	A_5	50	98.3	50	16.7±0.65
Natural rape, Lembke's		50	99.4	35	20.8±0.78
Mass-selection in a.n.o. 5	A_2	48	87.3	46	3.9±0.31
,, ,, ,,	A_3	49	87.9	48	4.9±0.54
,, ,, ,,	A_4	50	94.7	50	11.6±0.63
,, ,, ,,	A_5	50	95.6	50	16.9±0.56

TABLE 9. *Pollen fertility and number of seeds per pod in artificial and natural rape 1957.*

Variety	Number of plants	Pollen fertility per cent	Number of seeds per pod
Natural rape, Lembke's	25	98.4	22.1
A.n.o. 1	25	98.7	21.5
„ 3	25	97.5	20.3
„ 4	25	93.1	18.5
Sv 54/38 from a.n.o. 5	25	97.8	23.6
Sv 55/1069 „ „ 5	25	95.3	23.5
A.n.o. 7	25	93.3	22.2
Sv 55/1071 from a.n.o. 11	25	91.2	20.3
Sv 55/1072 „ „ 11	25	95.2	17.7
Sv 55/1073 „ „ 13	25	93.7	18.9
A.n.o. 17	25	96.9	22.1
Sv 56/1247 from a.n.o. 18	25	95.0	22.1
A.n.o. 19	25	96.6	21.8
Natural rape, Matador	25	97.6	20.8

The A_s material reported in Table 8 does not reach the number of seeds per pod obtained in populations of natural rape, tested at the same time and place. Continued selection has, however, further increased the number of seeds per pod. In addition, some of the artificial strains, derived from fertile A_1 plants, had from the very beginning a high number of good seeds per pod. Table 9 shows the number of seeds per pod in such artificial material and, as seen from this table, several artificial varieties have about the same number of good seeds per pod as the natural standard varieties. An analysis of variance of the data concerning number of seeds per pod, summarized in Table 9, gives no difference between natural and artificial rape ($v^2<1$), while there is a significant difference ($v^2=5.60^{***}$) between different artificial varieties, a result which gives good promise for the utilisation of this material in breeding work.

Some investigations on the degree of self-incompatibility in artificial rape and swede were carried out, a survey of the work with rape having been published earlier (OLSSON, 1953). For the present, therefore, it will only be mentioned that part of the artificial *napus* material was entirely self-compatible, *e.g.* a.n.o. 1—3, 7, 17, 19 and 22 and also a.n.r. 1—3 and 7. Another A_1 material was, however, strongly self-incompatible. This was best demonstrated in the fully fertile a.n.r. 6 (Fig. 8), but

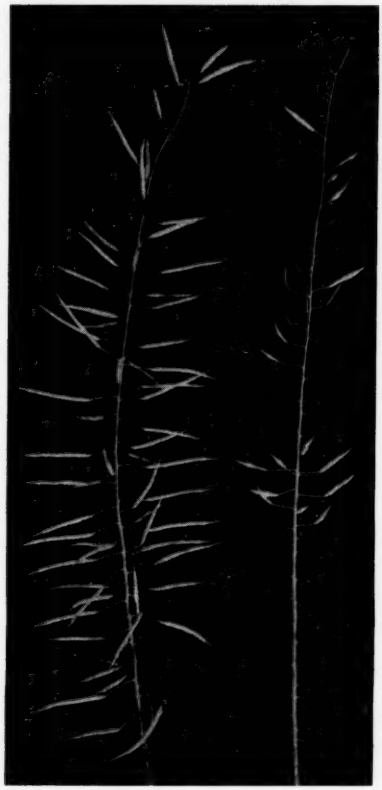


Fig. 8. Different degrees of self-compatibility in artificial *B. napus*. To the left self-compatible a.n.r. 7; to the right self-incompatible a.n.r. 6. Both branches self-pollinated, when cross-pollinated a.n.r. 6 is perfectly fertile.

even in partially sterile materials, such as *e.g.* a.n.o. 5, many A_1 plants were very markedly self-incompatible. Even in the material of artificial *B. juncea* earlier described both self-incompatible and self-compatible A_1 plants were obtained.

5. Characters of importance in practical agriculture

A. *Artificial rape*

It is hardly to be expected that the newly synthesized artificial rape will be equal to our best highbred varieties in all agronomic characters.

TABLE 10. *Relative yield of seed and oil, and some other characters of strains of artificial rape in relation to the natural varieties Matador or Lembke's winter rape.*

Variety	Number of trials	Relative value or difference from the standard (Matador or Lembke's rape)							
		Seed yield rel. value	Oil content % in dry matter diff.	Oil yield rel. value	Winter hardiness diff.	Date of maturity diff. in days	Lodging resist. diff.	1000 grain weight	
								Number of trials	Diff. gram
A.n.o. 1	8	85	−0.3	85	−0.6	+2	+0.3	5	−1.28
„ 3	3	66	−2.3	63	−4.1	+6	+0.7	2	−2.27
Sv 56/1235 from a.n.o. 4	2	84	−2.0	83	−0.3	+1	−0.9	—	—
Sv 54/38 „ „ 5	3	77	−0.8	76	−0.2	+3	−0.2	3	−0.21
Sv 55/1069 „ „ 5	8	107	−0.3	106	+0.4	+2	−0.9	8	+0.09
Sv 56/1236 „ „ 5	3	108	−0.4	107	±0.0	+1	−0.5	—	—
Sv 56/1238 „ „ 5	6	107	−0.1	107	+0.2	+1	−0.7	4	+0.05
Sv 58/1208 „ „ 5	2	115	+1.0	117	+0.7	±0	+0.6	1	+0.17
A.n.o. 7	7	87	−2.9	81	−0.3	±0	−0.1	4	−0.44
Sv 55/1071 from a.n.o. 11	4	86	−0.5	85	−1.5	+3	−0.2	4	+0.30
Sv 55/1072 „ „ 11	4	90	−2.0	86	+0.3	±0	−0.9	3	+0.41
Sv 55/1073 „ „ 13	7	87	−0.9	86	−0.4	+3	+0.3	6	+0.12
Sv 56/1247 „ „ 18	3	101	−0.6	100	−0.6	−4	−1.3	2	−0.94
A.n.o. 19	3	82	−0.1	82	−2.5	−4	−1.3	2	−1.51

Diff. between varieties in regard to winter hardiness and lodging resistance was calculated after grading these characters in the field according to a 10-degree scale, where 10 is the positive maximum.

TABLE 11. *Content of dry matter and sugar in terminal buds of some varieties of artificial and natural rape in the winter.*

Variety	Content of dry matter in per cent				Total amount of sugar (det. acc. to Somogyi) in per cent of dry matter			
	1954	1955	1957	Mean	1954	1955	1957	Mean
Natural rape, Lembke's	17.9	16.3	18.9	17.7	26.2	16.9	27.2	23.4
A.n.o. 3	15.6	14.0	19.7	16.4	21.8	14.7	27.7	21.4
Sv 55/1069 from a.n.o. 5	17.9	15.7	20.3	18.0	28.9	19.7	30.2	26.3
Sv 55/1072 „ „ 11	16.7	14.7	18.9	16.8	27.3	19.6	25.2	24.0
A.n.o. 17	16.9	15.3	18.6	16.9	24.7	18.6	27.4	23.6
„ 19	15.9	14.9	19.8	16.9	21.9	17.1	26.1	21.7
Natural rape, Matador	16.2	15.7	18.4	16.8	26.0	15.7	26.0	22.6

The new material is to be considered as a raw material for further breeding, and its greatest value lies in the possibility of introducing new genes from the primary species, *campestris* and *oleracea*, into the breeding material of *napus*.

In order to give an idea about the practical value of the new material, Table 10 summarizes some results obtained with artificial material, as far as known at present. The values of this table are related to the Matador winter rape, which is the variety most commonly grown in Sweden. In some of the trials, including a.n.o. 1, 3, 7 and Sv 54/38 from a.n.o. 5, the German variety Lembke's winter rape was used as standard. This variety is closely related to Matador and only 3 per cent inferior in yield.

As seen, the yield of the majority of the artificial varieties is about 15 per cent inferior to that of the best market varieties, a difference which corresponds fairly well to the difference between land varieties and highbred ones (*cf.* data quoted by ANDERSSON and OLSSON, 1959, p. 48). In several cases the low yield can be attributed directly to low winter hardiness, since, after mild winters, some of the artificial varieties have given yields well comparable to those of the standard varieties. Some of the strains descended from a.n.o. 5 stand out markedly from the rest of the artificial material and exceed Matador in seed yield with 7—8 per cent. The strain most thoroughly tested, Sv 55/1069, gave in 8 trials at Svalöf on an average 329 ± 78.8 kg. per hectar more than Matador ($t = 4.18^{**}$).

As a rule, the new artificial material has a lower oil content than the standard varieties, but selection has indicated that it is possible to in-

crease the oil content in the material, and several new strains from a.n.o. 5 now exceed Matador in oil content. Sv 58/1208 is quoted as an example in Table 10. Seed size is larger in some varieties, smaller in others, than in Matador; a.n.o. 1, 3 and 19 have strikingly small seeds.

The winter hardiness was scored according to a 10 graded scale, with 1=totally killed and 10=undamaged. As a rule, the artificial material was inferior to Matador, and especially a.n.o. 3, 17 and 19 showed a very poor hardiness. On the other hand, several of the strains derived from a.n.o. 5 proved somewhat superior to Matador in hardiness, and these differences were verified in freezing experiments. In analyses of overwintering leaves and stem buds, made by the chemical laboratories at Svalöf, Sv 55/1069 from a.n.o. 5 showed a somewhat higher content of dry matter and sugar than other varieties analysed. The results of some of these analyses are given in Table 11.

According to TORSSELL (1959), it is favourable for overwintering if the epicotyle is short throughout the winter so that the vegetation point will be as close to the soil surface as possible. There are considerable differences between artificial varieties in the degree of elongation of the epicotyle. These differences become especially apparent when the material is sown early, and the results of such an experiment are shown in Table 12. A.n.o. 3, 17, 19, Sv 55/1071 from a.n.o. 11 and Sv 56/1247 from a.n.o. 18 are varieties with a marked tendency to elongation in the autumn. From a practical point of view it is very interesting that Sv 55/1072 has a very short epicotyle and the vegetation point near the soil surface, and this in spite of the fact that it grows with normal rapidity in the autumn. Unfortunately, Sv 55/1072 has a low yield and low oil content, but crosses are now being made with this strain in order to introduce its favourable mode of growth into other varieties.

Also in some other practically important characters, the artificial material offers certain advantages. Several of the artificial strains grow more rapidly than Matador when sown late in the autumn. Strains from a.n.o. 5 and a.n.o. 11 and crosses between these and natural rape are more resistant than Matador to attacks by *Peronospora* in the seedling stage.

In addition, the artificial material was tested as fodder rape, and some of the data from these trials are summarized in Table 13. As seen from the table Sv 55/1069 from a.n.o. 5 and Sv 55/1071 from a.n.o. 11 produced more dry matter than the varieties Matador and Vestal, and Sv 55/1071 exceeded Matador in dry matter yield in 17 trials with on an average 556 ± 88 kg. per hectare ($t=6.32^{***}$). The protein content of the

TABLE 12. *Height of the vegetation point above soil surface in winter after early sowing in the autumn.*

Variety	Number of plants	Height of the vegetation point in cm above soil surface $\bar{x} \pm m$
Natural rape, Matador	50	7.7±0.29
Sv 55/1069 from a.n.o. 5	50	6.3±0.28
Sv 56/1238 „ „ 5	50	7.0±0.18
Sv 55/1072 „ „ 11	50	4.3±0.22
Sv 55/1073 „ „ 13	50	9.0±0.22
Sv 56/1247 „ „ 18	50	13.3±0.38

artificial material was somewhat lower than that of the standard varieties, but nevertheless Sv 55/1071 yielded more protein per hectare than Matador or Vestal. The English fodder rape Garton's Early Giant, which cannot be grown for seed in Sweden because of frost sensitivity, has a higher yield of dry matter than the artificial varieties, but the protein content is lower and the content of crude fibre higher, and thus the quality of the crop lower than that of the other varieties tested.

Crosses between artificial and natural rape have been carried out on a large scale, the two groups of material intercrossing easily and giving fully fertile hybrids (Fig. 9). The artificial material may thus be used in the breeding work to introduce new genic material in the best varie-

TABLE 13. *Some data from the testing of artificial rape as a fodder crop.*

Variety	Number of trials	Dry matter			Crude protein % of dry matter	Crude fiber % of dry matter
		%	Kg. per hectare	Rel. value		
Natural rape, Matador	17	14.2	4557	*100*	16.1	14.7
Sv 55/1069 from a.n.o. 5	17	14.5	4678	103	15.7	14.1
Sv 55/1071 „ „ 11	17	13.5	5113	112	15.1	14.8
Natural rape, Matador	5	12.8	4150	*100*	16.3	16.0
„ „ , Vestal	5	12.6	4170	100	17.6	15.1
Sv 55/1069 from a.n.o. 5	5	12.6	4240	102	15.9	14.4
Sv 55/1071 „ „ 11	5	12.7	4930	119	15.2	15.2
Natural rape, Early Giant	5	12.5	5340	129	14.5	20.2

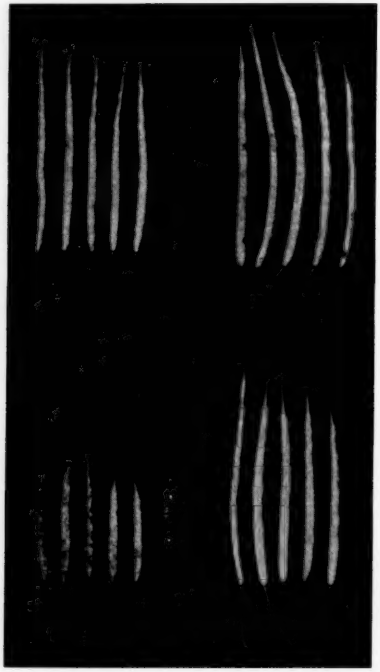


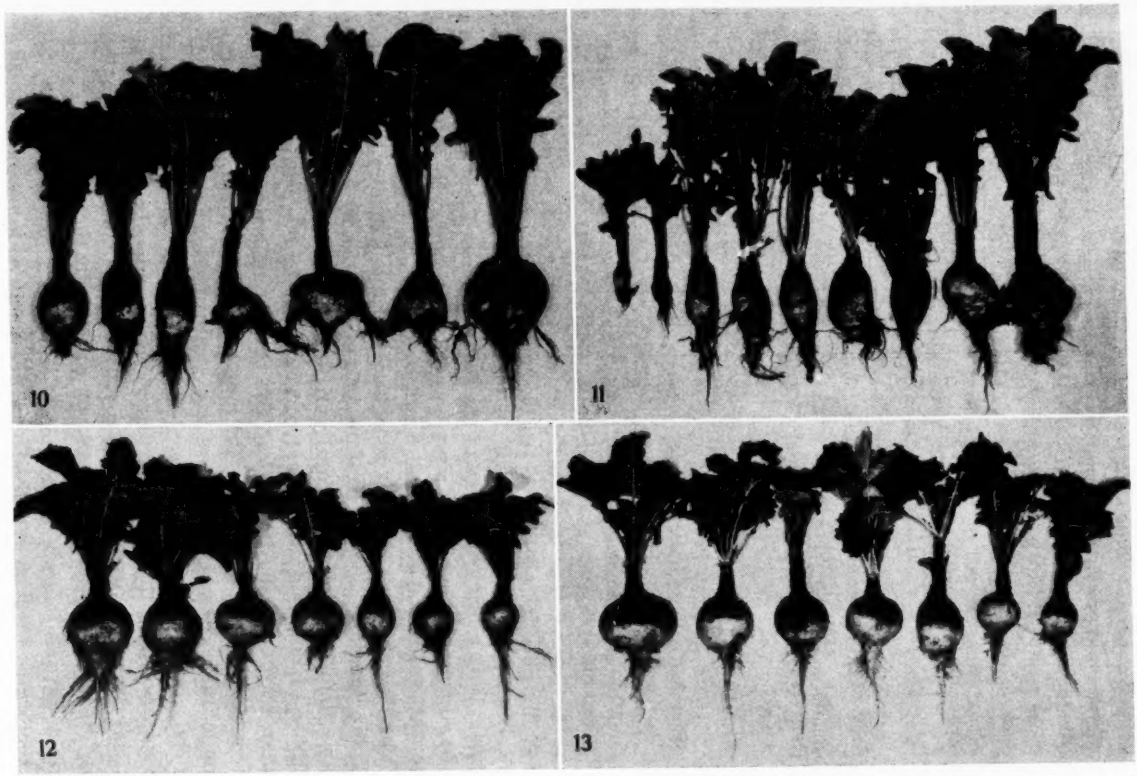
Fig. 9. Above: to the left pods from a.n.o. 1; to the right pods from a.n.o. 1×natural rape. — Below: to the left pods from a.n.o. 2; to the right pods from a.n.o. 2×natural rape.

ties available at present. That such combinations may result in obvious success has already been shown by RUDORF (1958).

The observations concerning the practical value of artificial rape may be summarized as follows: Most of the material is inferior to the best market varieties, but several lines from a.n.o. 5 are superior to Matador in yield of seed and oil, and at the same time have a somewhat better winter hardiness, grow more rapidly, and have a better resistance to *Peronospora*. It is, thus, possible, by simple selection in the artificial material to obtain varieties of direct practical value. By crossing different artificial strains with one another or with natural rape still further improvements may be obtainable.

B. *Artificial swedes*

The artificial material of swedes as well as crosses between this mate-



Figs. 10—13. Artificial and natural swedes. Fig. 10. A.n.r. 1. Fig. 11. A.n.r. 4×1. Fig. 12. A.n.r. from a population of (1×4)×(1×5)×(4×1)×(5×1)×(4×3)×(5×3). Fig. 13. Natural Fenix-Bangholm.

TABLE 14. *Some data from the testing of artificial swedes.*

Variety	Number of trials	Dry matter			Crude protein % of dry matter	Stem length in mm
		%	Kg. per hectare	Rel. value		
Natural swede, Viktoria	3	11.7	7890	*100*	10.7	34
„ „ , Fenix-Bangholm	3	10.8	8840	112	9.7	50
A.n.r. 1	3	10.4	6240	79	11.7	74
„ 2	3	10.5	7555	96	11.0	45
„ 3	3	11.0	6440	82	10.2	52
Natural swede, Viktoria	2	11.8	9110	*100*	9.8	44
„ „ , Fenix-Bangholm	2	11.2	10590	116	9.2	59
A.n.r. 7	2	11.5	6795	75	9.7	74
Natural swede, Viktoria	10	11.9	7945	*100*		
„ „ , Fenix-Bangholm	10	11.3	8590	108		
(A.n.r. 2×Fenix)×(A.n.r. 1×Fenix)	10	11.6	8505	107		
Natural swede, Viktoria	5	12.5	8120	*100*		
„ „ , Fenix-Bangholm	5	12.1	8780	108		
A.n.r. 2×Bangholm	5	12.4	8695	107		

rial and different natural varieties, have been tested in field trials by A. JOSEFSSON, Svalöf, and will be dealt with by him in other connections. Only a brief summary of the results obtained will be given here. On an average the roots of a.n.r. 3 and 7 were smaller than those of the market varieties Viktoria and Fenix Bangholm, whereas those of a.n.r. 1 and 2 were larger than the roots of Viktoria but smaller than those of Fenix Bangholm. The roots of the artificial swedes were also more variable than those of the natural varieties (Figs. 10—13). As seen from Table 14 the yield of dry matter was lower in the artificial material than in the standard varieties. The content of crude protein proved remarkably high in a.n.r. 1. The long leaf stock of a.n.r. 1 and 7 is an undesirable character. The best new strains from crosses between artificial and natural swedes are about on a level with the best natural varieties. As in the case of artificial rape the newly synthesized artificial strains of swedes should be considered as valuable raw material for further breeding and not as new varieties by themselves.

6. Cytology

Earlier studies of the cytology of artificial *B. napus* have shown that the 38 chromosome hybrid as a rule has a regular meiosis with 19_{II},

while some cells occur with $18_{II}+2_{I}$ or with $17_{II}+4_{I}$ (U, 1935; FRANDSEN, 1947; RUDORF, 1950). The present author has not had the opportunity to make a detailed study of the cytology of the artificial *napus* material here presented, which will instead be dealt with in a special paper from the cytogenetic department at Svalöf. Preliminary studies have shown, however, that the meiosis is much more regular in the artificial *napus* material than in the *juncea* material earlier dealt with, the meiosis of the fully fertile A_1 plants generally being as regular as in the natural species.

The meiosis of the 19 chromosome plant a.n.r. 9 agreed largely with that of spontaneous haploid rape (OLSSON and HAGBERG, 1955), but the frequency of bivalents was somewhat higher and several PMC:s showed $9_{II}+1_{I}$.

IV. DISCUSSION

As already pointed out in the present paper, as well as in an earlier paper on artificial *B. juncea* (OLSSON, 1960), *B. carinata* BRAUN (with the genome constitution bbcc), *B. juncea* COSS. (aabb) and *B. napus* L. (aacc) are amphidiploids between *B. nigra* KOCH (bb), *B. oleracea* L. (cc) and *B. campestris* L. (aa). The amphidiploids may be artificially synthesized by crosses between the parents on the diploid as well as on the tetraploid level. Disregarding complications, caused by irregularities at meiosis of the tetraploids, the amphidiploid may be obtained directly by crossing the tetraploids. If diploids are intercrossed, not only will the expected diploid hybrids appear but also some amphidiploids and different triploid combinations. The crosses between the diploids are of special interest since natural *carinata*, *juncea* and *napus* must have originated in this way. For this reason, the possibilities to obtain amphidiploids from crosses between diploids in the genus *Brassica* will be discussed at some length.

In the *Brassica* species under consideration, meiosis and gamete formation are almost completely regular, and normal fertilisation is necessary for the formation of embryos. Exceptionally, however, a parthenogenetic development of the haploid egg cell may occur, probably after same kind of pseudogametic stimulation. This process will, at least occasionally, result in spontaneous haploid plants (OLSSON and HAGBERG, 1955). If the haploid egg cell, stimulated by pseudogamy, doubles its chromosome number, the result will be a diploid matromorphous plant; at least some of the matromorphous plants which are frequent after interspecific pollination, may have such an origin (NOUGUCHI,

1928; U, 1935; MOHAMMAD and SIKKA, 1940; RAMANUJAM, 1940). If the doubling does not occur at the first division of the egg cell but later on, haploid-diploid sectorial chimaeras may originate (Fig. 14). If the sperm nucleus instead of disintegrating after having stimulated the egg to divisions, fuses with the egg cell after this has doubled its chromosome number, the result will be a triploid with two genomes from the mother and one from the father. U (1935) is of the opinion that his hybrid COF_1-III was derived in this manner, and in the material of the present author the 28 chromosome hybrid between *campestris* and *nigra*, as well as the 29 chromosome hybrids between *campestris* and *oleracea* may have such an origin. If the fertilisation is followed by a chromosome doubling, however, the result will be an amphidiploid hybrid. U (1935) believes that his hybrid COF_1-IV was formed in this way; among the hybrids dealt with here, the a.n.o. 19, originally with 19 chromosomes, has doubled its chromosome number somatically at a somewhat later stage.

U (1935) considers it unlikely that the diploid primary species are capable of producing unreduced gametes. Consequently, he interprets the cases in which his triploid hybrids contain one genome from the mother and two from the father, as due to double fertilisation. With our present knowledge, however, it seems probable that the natural species, too, produce unreduced gametes in a low frequency. According to FUKUSHIMA (1931) doubled PMC:s occur in *B. "japonica"*, which belongs to the *campestris* complex. Also the statements of MORINAGA and KURIYAMA (1937) concerning the occurrence of restitution nuclei and the formation of dyads instead of tetrads in *napus*, support this idea. MIZUSHIMA (1952) considers it possible that matromorphous plants may be formed from parthenogenetically developed unreduced egg cells, as well as from haploid eggs in the manner already described. The presence of unreduced gametes may even explain the appearance of hybrids with one supernumerary genome (RUDORF, 1950; MIZUSHIMA, 1952), and in the material studied here, the 28 and 29 chromosome hybrids (aab and aac) already mentioned may have developed from unreduced *campestris* egg cells, too. The differences in chromosome number in the reciprocal crosses of *juncea*×*campestris* are understood most easily by the assumption that unreduced gametes function in *campestris*. In the cross *juncea* (aabb) ♀×*campestris* (aa) ♂, 113 plants had 2n=28 (aab) and one plant 2n=38 (aaab), while the reciprocal cross gave 37 plants with 2n=38 (aaab) and one with 2n=28 (aab). When *campestris* is used as father, fertilisation will mainly be by haploid pollen, since un-

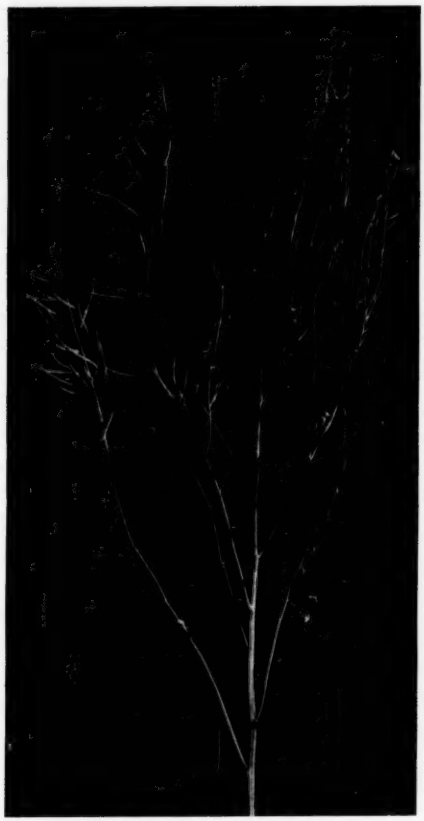


Fig. 14. Haploid chimaèra of rape.

reduced gametes on the male side probably have less chance to function, haploid pollen tubes as a rule reaching the egg faster than the diploid ones (*cf.* JULÉN, 1950). On the other hand, when *campestris* is used as mother, the unreduced eggs, which have about the same chromosome number as the male gametes, are favoured, either immediately at the fertilisation or later, during the development of the zygotes. If unreduced gametes from both parental species form a zygote, the amphidiploid is directly formed.

It is thus possible that the natural amphidiploid species has been formed directly from crosses between diploid forms; a chromosome doubling may takes place at the first division of the zygote or later,

giving a homozygote, or the hybrid originates by the fusion of unreduced gametes, resulting in a heterozygote. Again, the first hybrid may have been a fairly sterile diploid plant. Since such a plant will produce unreduced gametes, amphidiploids will be formed in F_2, and they will be homozygous if they are the result of selfing. Finally, the synthesis may happen *via* triploid F_1:s of the constitutions aac or acc.

In their work with synthesis of *B. napus* HOFFMANN and PETERS (1958) were unable to show differences in crossability between individual plant. MIZUSHIMA (1952), however, is of the opinion that there are considerable such differences between different plants within a *Brassica* species, and his opinion is further confirmed by the present results. The differences in crossability between plants of allogamous *Brassica* species correspond to those found by BERNSTRÖM (1955) between pure lines of *Lamium* and which, according to OEHLER (1958), is of common occurrence.

Morphologically, the artificial material of *B. juncea* and *B. napus* agrees very well with natural material, and in many cases no differences are observed between the newly synthesized forms and the natural species. In other cases, the new material differs from natural material only in some taxonomically unimportant character, and only seldom the new material deviates enough from natural *napus* as to be intermediate between this species and *campestris*. The great morphological similarity between the new artificial material and the natural species indicates that the latter is of fairly recent origin and may derive from still existing types of the primary species.

B. napus can have originated spontaneously only in regions where *B. campestris* and *B. oleracea* were growing together. According to SINSKAIA (1928), wild *B. campestris* occurs from western Europe to eastern Asia, while wild *oleracea* grows along the Atlantic coast of western Europe and in the Mediterranean region. Chances for the spontaneous synthesis of *B. napus* should, thus, occur within the last mentioned regions, which agrees well with SINSKAIA's and SCHIEMANN's ideas that *B. napus* is a South-west European-Mediterranean species (SINSKAIA, 1928; SCHIEMANN, 1932). Although *B. campestris* and *B. oleracea* do not cross very easily, the difficulty with which they may be crossed it not greater than that *B. napus* may be assumed to have originated several times, at different places and from different types of the parental species (RUDORF, 1950; OLSSON, 1954).

According to SCHIEMANN (1932) the *rapifera* form of *B. napus* should have been developed from the *oleifera* form by selection. In the crosses

here reported, it has been shown that *napus rapifera* may be synthesized directly from crosses between *oleracea* and *rapifera* forms of *campestris*. It is, therefore, likely that the material from which our present swedes have developed had a swollen root already from the beginning. That no wild form of *B. napus* is known may depend upon the difficulty in distinguishing between a true wild form and escapes from cultivation, or upon the eradication of the true wild type. It is also possible, however, that *napus* has originated in the gardens of our forefathers, where turnip and kale were growing side by side. At least it is rather tempting to assume such an origin for the swede.

B. nigra shows a great variability in the eastern Mediterranean and neighbouring regions, while *B. juncea* occurs from Abyssinia to eastern Asia (SINSKAIA, 1928; SCHIEMANN, 1932). In view of the wide variability occurring within *juncea*, it seems most probable that it has been generated at different places within the region of *B. nigra* and with different forms of the parents partaking in the synthesis.

Some of the A_1 plants of *B. napus* and *B. juncea* here dealt with were fully fertile, while others had more or less strongly reduced fertility. In most cases, continuous selection within the sterile material has resulted in full fertility, however. Reduced fertility in the first genrations of newly synthesized species, followed by a gradual increase in fertility in the following generations, has been reported in several materials (CLAUSEN, KECK and HIESY, 1945; GAJEWSKI, 1946; *cf.* also STEEBBINS, 1950). A part of the improved fertility in artificial *juncea* is caused by the meiosis becoming more regular in later generations. On the other hand, A_1 plants with fairly regular meiosis often had a strongly reduced fertility. Thus, a reduction in seed setting is no doubt caused also by other factors than chromosomal disturbances. A considerable part of the sterility is probably due to unsatisfactory interaction between the genomes brought together, causing anatomical and physiological disturbances (*cf. e.g.* CLAUSEN, KECK and HIESY, 1945; STEBBINS, 1950; BERNSTRÖM, 1955). The improvement in fertility in later generations is probably the result of a more regular meiosis and a balanced interaction between the genomes, in their turn caused by genic recombinations. There is, even in the natural species, a certain tendency towards a stronger differentiation of the genomes, visible in a somewhat lower frequency of bivalents in amphihaploids derived from natural amphidiploids than in corresponding diploid F_1 hybrids (*cf.* RAMANUJAM and SRINIVASACHAR, 1943; MIZUSHIMA, 1950 b; OLSSON, 1960).

As a rule, the natural amphidiploid species, *B. juncea* and *napus*, are

self-compatible, while the parental species are mainly self-incompatible. In the author's material of artificial *napus* and *juncea*, some plants proved to be self-incompatible, others self-compatible. U:s artificial *napus* was self-compatible, while material (*i.a. juncea* and *carinata*) studied by MIZUSHIMA (1950 b, 1952) and MIZUSHIMA and KATSUO (1953) as a rule was self-incompatible. Self-compatibility is not caused by amphidiploidy as such, but is controlled genetically. It is probable that when *napus* and *juncea* have been spontaneously synthesized in nature only one or a few hybrid plants were formed each time. If these were self-compatible, then stood a better chance of reproduction, and their risk of back crossing with the parents would be less than if they were self-incompatible. Consequently, at least a certain degree of self-compatibility is favourable for the survival of newly synthesized forms, and since self-compatible plants are most likely favoured in the first selections, the amphidiploid species will become mainly self-fertile.

When judging the practical value of the new forms, they should be considered as raw material for further breeding rather than as finished varieties. A fully fertile new form is comparable to an unbred land variety; partly sterile new types must be bred for some time even to reach this level. The greatest potential value of the artificial material is the widening of the genic variation it brings into the amphidiploid species. Before experimental synthetic work had started, the rape breeder was restricted to the variation existing within natural *B. napus* and to incidental mutations; now it is possible to utilize for breeding work the wide variation within *campestris* and *oleracea*. Only those forms of *oleracea* and *campestris* that happened to grow near each other could possibly take part in natural syntheses — man can overcome this isolation and create a much wider variation within *napus*.

When synthesizing *e.g.* rape, it would be reasonable to choose parent varieties with characters, desired in the synthetic product, and such a choice may sometimes be at least partly successful. In most cases, however, it is impossible to judge the combining capacity of a variety from its own value. Would any breeder, for instance, attempt to improve tetraploid wheat by crossing it with *Aegilops squarrosa*, had not nature already shown the value of such a combination? It will therefore be advisable to synthesize a large artificial material, using different subspecies and varieties of the parent species. The artificial material should then be further handled by conventional breeding methods, with special emphasis on recombinations between different artificial materials, and between such materials and natural varieties.

RUDORF (1950, 1958) has already shown that selection from crosses between natural and artificial rape may result in varieties with an oil yield, 7 per cent higher than that of Lembke's rape. Simply by selection within artificial material, it was possible, in the present work, to breed varieties showing about the same superiority over Matador, which under Swedish conditions has somewhat better yield than Lembke's rape. Other valuable characters found in the artificial material at Svalöf are increased resistance to *Peronospora*, relatively good ability to grow when sown late and a low vegetation point during winter.

The attempts to introduce new genes into the amphidiploid species of *Brassica* should not be confined, however, to new syntheses only. Back crosses with the primary species should offer possibilities to obtain valuable new combinations. Finally, there are, within the genus *Brassica*, great possibilities to create completely new species, either as digenomic hexaploids (FRANDSEN and WINGE, 1932; CHOPINET, 1942, 1943; MIZUSHIMA, 1950 b) or as trigenomic hexaploids (HOWARD, 1942; MIZUSHIMA, 1950 b; IWASA, 1951). The extensive experiences of MIZUSHIMA, as well as experiences gained from work at Svalöf indicate, however, that, from practical point of view, species are much more promising on the tetraploid level than on the hexaploid level.

V. SUMMARY

(1) Artificial *B. napus* has been synthesized by crosses between *B. campestris* and *B. oleracea*. The crosses were made on the diploid as well as on the tetraploid level.

(2) Both ssp. *oleifera* and ssp. *rapifera* of *B. napus* were synthesized.

(3) The artificial *napus* material was either morphologically indistinguishable from natural material, or, sometimes, distinctly different in one or a few characters.

(4) The newly synthesized *napus* material was often completely fertile, but in several cases the fertility of the new material was strongly reduced. Selection for a few generations in material of the latter kind, resulted in fully fertile strains.

(5) The newly synthesized forms of rape and swedes were inferior to the best market varieties in yield of oil and roots, respectively. By simple selection in the artificial rape material, however, varieties were obtained which outyielded the most commonly grown variety Matador with about 7 per cent. In the material of swedes, hybrids between artificial and natural varieties gave yields comparable to the high yielding variety

Fenix-Bangholm. The synthesis of new *napus* forms makes it possible to introduce genes from the primary species *campestris* and *oleracea* into *napus*, and thus to increase the variability of this species.

(6) Different problems in relation to the spontaneous synthesis of the amphidiploid species, and to the practical value of the artificial material are discussed.

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INHERITANCE STUDIES IN LETTUCE

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I. INTRODUCTION

THE garden lettuce, *Lactuca sativa* L, exhibits an unusual degree of variation in morphological characters. This polymorphism provides good scope for genetical studies and this scope is further increased by the fact that hybrids with the closely related wild species *L. serriola* TORNER (called by many authors *L. scariola* L), are easily obtained and highly fertile. An account of the cytology and fertility of *L. sativa*, *L. serriola* and their hybrids has been given by the writer in another paper (LINDQVIST, 1960 a), where it has been shown that they must be considered as belonging to the same ecospecies. The garden lettuce has one serious disadvantage as a genetical material, namely that for good vegetative development it requires cool and moist conditions, but for seed production dry conditions are essential. In England, where this work has been carried out, the climate is usually suitable for vegetative development but rarely for ripening of the seed.

Several authors have published reports of genetic studies in *L. sativa* and *L. serriola*. References to these reports, and discussion of the results are found under various headings in this paper. The work to be reported here was undertaken partly with a view to gaining further knowledge about the relationship between *L. sativa* and *L. serriola*, and partly in the hope of obtaining results which could be of use in practical breeding work.

II. MATERIAL AND METHODS

The lines used in this study originate from *L. serriola*, *L. sativa* and segregates from various crosses within or between these two species. The material of *L. sativa* included some presumed primitive forms (LINDQVIST, 1960c). The majority of the lines have been used in investigations reported in earlier papers (LINDQVIST, 1956, 1958 and 1960 a), to which the reader is referred for full details and also for an account

of the methods of cultivation, crossing etc. The following lines used in the present investigation have, however, not been mentioned earlier:

B. Bost.	from	Big Boston, commercial cabbage lettuce variety
Cont.	„	Continuity (Crisp as Ice), commercial cabbage lettuce variety
Chesh.	„	Cheshunt Early Giant, „ „ „ „
Laib.	„	Laibacher Eis (Iceberg), „ „ „ „
Montrée	„	Montrée jaune frisée à bords rouges, commercial lettuce variety
V. grasse	„	Verte grasse, commercial cabbage lettuce variety
R. verte	„	Romaine verte d'hiver Royale, commercial self-folding cos lettuce
Gr. 10	„	R. verte × Merv.
IIGG	„	R. Liebl. × V. grasse
IIgg	„	„ „
iiGG	„	„ „
Tinged	„	K. driv × Laur.
tt tete	„	R. verte × Tezier

In the statistical treament of the data, χ^2's for deviation and heterogeneity have been designated by suffixes which are generally self-explanatory. Thus $\chi^2_{3:1}$ means a χ^2 for deviation from a 3 :1 ratio, χ^2_L means a χ^2 for linkage and $\chi^2_{\text{het. crosses}}$ a χ^2 for heterogeneity between crosses. Figures in brackets after the χ^2 refer to the number of degrees of freedom. Accordingly $\chi^2_{L(5)}$ would mean a χ^2 for linkage with 5 degrees of freedom. The significance of χ^2's has been indicated with asterisks, * for significance at the 5 % level, ** at the 1 % level and *** at the 0.1 % level; χ^2's without any asterisks correspond to P-values bigger than 5 %.

The BRANDT and SNEDECOR formula (MATHER, 1957) has been used for calculation of χ^2 for heterogeneity in cases when there was a serious deviation from expected ratios. A formula based on the square root transformation of the POISSON distribution has been used for testing homogeneity in cases where expectation in the recessive class was too low for the ordinary χ^2 formula. This formula, which has been suggested by Docent G. BLOM, Lund, reads as follows:

$$\chi^2 = S\left(\sqrt{a_i} - \sqrt{n_i m}\right)^2$$

where a_i is the number of recessives, n_i the total number of plants in each family and m is $\frac{Sa_i}{Sn_i}$. For the purpose of calculation this formula is conveniently written: $\chi^2 = 8\left(Sa_i - \sqrt{m}\, S\sqrt{a_i n_i}\right)$.

When single gene ratios were disturbed the formula given by MATHER (1949, p. 187) was used for detection of linkage. The maximum likelihood method has been used for various estimations. FISHER's scoring

method (FISHER, 1946, MATHER, 1957) has been used for calculation of recombination fractions and testing of homogeneity. This method is applicable also if one single gene ratio is disturbed, if the loss of information is allowed for by using the following formula, suggested by Dr. M. J. LAWRENCE of Birmingham, for the amount of information, I:

$$I \text{ (for coupling phase)} = \frac{2n(1-p)}{3+v} [D_1 + (1+v)D_2 + D_3]$$ where v is the viability fraction of the gene concerned and D_1, D_2 and D_3 the scores, neglecting signs, in the tables by FISHER and YATES (1948, p. 58) for the classes of the double dominants, single recessives and double recessives respectively. Families in which both single gene ratios were disturbed have not been used for calculation of crossing over values.

III. EXPERIMENTAL RESULTS

The inheritance of a number of characters affecting various stages of the plant development have been studied. It has not been considered necessary to publish all results from individual crosses. Data on F_2-segregations attributable to single genes have been summarized in Table 1. Each character or character complex will be considered separately and further data presented were necessary.

For each gene in Table 1, reference has been made to the paper in which it was reported for the first time; where there is no reference the gene was not known previously. The gene symbols used in Table 1 do not always agree with those used by the original authors, as it has been necessary in some cases to change the designations in order to avoid confusion. More than two alleles have been involved in the segregations at the *R* and *Sp* loci. In these cases the data in Table 1 are a summary of all segregations observed at the locus.

1. Anthocyanin characters

Variation and previous results. — Anthocyanin pigmentation shows great varietal variation in lettuce. Some varieties have only a slight tinting at the leaf margin, others have in addition numerous dark purple spots and yet others a dark, reddish brown pigmentation of the whole lamina. THOMPSON (1938), in his genetic study of pigmentation in lettuce, referred to these types as the 'tinged', 'spotted' and 'red' types. He found that two complementary, dominant genes which he called *C* and *T* are necessary for the production of anthocyanin. In the presence of these

TABLE 1. *Summary of single gene ratios from F_2-families.*

Character affected	Gene	Author	Dominant	Recessive	Total	$\chi^2_{3:1}$	Heterogeneity between crosses		Heterogeneity between families	
							χ^2	DF	χ^2	DF
Anthocyanin colour	*C*	THOMPSON, 1938	243	91	334	0.898	—	—	5.268	4
" "	*T*	DURST, 1930	8589	2842	11431	0.116	56.728*	37	78.953	85
" "	*R*	THOMPSON, 1938	3098	1067	4165	0.849	32.295*	20	48.646	40
" "	*I*		4544	1163	5707	65.009***	133.680***	23	46.511	38
" "	*V*		1713	338	2051	79.409***	40.631***	6	8.126	17
Leaf colour	*G*	BREMER, 1931	7743	1995	9738	105.791***	161.264***	24	88.834	74
" "	*Lg*	THOMPSON, 1938	2314	506	2820	74.895***	51.175***	11	25.115	19
Leaf apex	*P*		3437	1117	4554	0.541	5.708	16	20.855	40
Venation	*St*	WHITAKER and BOHN, 1953	534	144	678	5.115	1.830	2	0.341	2
Waxiness	*Gl*		1398	452	1850	0.318	8.729	5	12.844	12
Reaction to photoperiod	*L*	BREMER, 1931	3093	1064	4157	0.786	8.327	13	40.786	30
Growth habit	*K*	BREMER and GRANA, 1935	440	124	564	2.733	—	—	6.940	5
" "	*H*		1814	556	2370	2.998	11.210	9	19.470	19
Spination	*Sp*	DURST, 1930	3604	1124	4728	3.795	16.893	16	39.424	38
Seed colour	*W*	DURST, 1930	3291	1015	4306	4.685*	25.451	19	23.965	38
" "	*Y*	THOMPSON, 1943	758	246	1004	0.133	8.408	5	1.207	6

two fundamental genes, the allele *R* produces the 'red' type, *r'* the 'spotted' type and *r*, the bottom recessive of the series, produces the 'tinged' type. These three alleles also control the pigmentation of the involucral bracts, which shows a variation similar to that of the pigmentation of the leaves, but they do not affect the anthocyanin colouring of the stem.

The majority of varieties of garden lettuce which lack anthocyanin have the genotype *CCtt*. THOMPSON (1938) proved that the variety New York (syn. Wonderful) is an exception as it has the genes *ccTT*. The recessive gene *c* has not been identified in any other variety of *L. sativa*, but it may be mentioned in this connection that the writer has obtained anthocyanin coloured F_1-hybrids between the anthocyanin-free *L. virosa* line, referred to as Vir. L (LINDQVIST, 1960 a) and the anthocyanin-free *L. sativa* lines Asp., Berl. and SL. This indicates that the line Vir. L probably has the genotype *ccTT*, *i.e.* the same as the variety New York.

ERNST-SCHWARZENBACH (1936) reported dihybrid F_2-ratios in crosses between the anthocyanin-free variety Gelber krauser Schnittsalat and the dark red varieties Wunder der 4 Jahreszeiten and Blutroter Kochsalat. It is therefore possible that the leaf lettuce variety Gelber krauser Schnittsalat has the genotype *cctt*. Another case of deviation from a 3 : 1 ratio was found by DURST (1930) in a cross between Paris White Cos and non-lobed *L. serriola*, which gave a large excess of non-pigmented plants. The line from Paris White Cos used by the writer has, however, always given the results to be expected if it carries the genes *CCtt*.

THOMPSON (1938) also tested a form of *L. serriola* with regard to anthocyanin genes, and found that it belonged to the 'tinged' type, having the genotype *CCTTrr*. This has also been the case with all forms of this species observed by the writer. ERNST-SCHWARZENBACH (1936), however, worked with a form of *L. serriola*, which lacked anthocyanin.

MC COLLUM (1953) described an anthocyanin character which he called 'dotted involucre' and which may be inherited as a simple recessive.

Own results. — The results in Table 1 show for the genes *T* and *R* a certain heterogeneity of the data. This is due to rather large deviations in a few crosses, but these abnormalities have not been fully analyzed.

In the course of this investigation three more genes affecting anthocyanin colour have been encountered, namely *r''*, which is a new member of the *R*-series, *i*, a recessive, which extends and intensifies the pigmentation produced by *C* and *T*, and finally *v*, a recessive which has

TABLE 2. *Evidence that r″ is allelic to R, r′ and r.*

Crosses	Parental genotypes	F_1	F_2							
			Number of fam.	Segregation						*R*-alleles	
				R	*r″*	*r′*	*r*	*tt*[1]	Total	$\chi^2_{3:1}$	$\chi^2_{het.}$
Laur.×Merv.	*TTr′r′×TTRR*	*R*	5	220	—	76	—	—	296	0.072	7.747
K.driv×Merv.	*ttrr×TTRR*	*R*	3	194	—	—	62	78	334	0.083	2.616
K.driv×Laur.	*ttrr×TTr′r′*	*r′*	2	—	—	94	36	31	161	0.503	0.000
Oakl.×Merv.	*ttr″r″×TTRR*	*R*	3	156	59	—	—	73	288	0.684	2.397
Laur.×Oakl.	*TTr′r′×ttr″r″*	*r″*	1	146	54	—	—	61	261	0.427	—
Tinged×Oakl.	*TTrr×ttr″r″*	*r″*	1	71	—	—	26	35	132	0.168	—

[1] *tt*-plants lack anthocyanin.

an opposite effect. In *vv*-plants the anthocyanin colour, produced by *C* and *T* vanishes gradually as the plants develop.

Anthocyanin pigmentation in lettuce is greatly influenced by environmental factors. Conditions unfavourable for vegetative development, *e. g.* drought and low temperatures, tend to increase the amount of anthocyanin. This must, of course, be taken into account when classifying for these characters.

r″, a new allele of the R-series. — This gene was first met in crosses between Oakl., a line which lacks anthocyanin, and tinged and spotted varieties. The F_1-hybrids were intermediate between the two types 'spotted' and 'red'. Spots were present on the leaves and in addition there was a pigmentation similar to that of the type 'red', but weaker in degree and more limited in extent. The new type is under normal conditions more difficult to distinguish from 'spotted' than from 'red', but the difference can be seen plainly on young seed stalks or side shoots. The leaves have at the tips of such shoots a brownish red anthocyanin colour similar to that of the 'red' type but weaker. This colouring is not found in the 'spotted' type. On older leaves it is much less pronounced than in the 'red' type, often completely absent.

F_2-data from crosses between Oakl. and 'red', 'spotted' and 'tinged' types are found in Table 2. The first three crosses in this table prove that Merv., Laur. and K.driv each have a different allele of the *R*-series, and the last three crosses prove that *r″* is also a member of this series. As both Oakl. and K.driv are recessive for the gene *t*, and consequently lack anthocyanin, the latter was in the last cross in Table 2 replaced by the line Tinged, selected from the cross K.driv×Laur. The F_1-results show that the new allele is recessive to *R*, but dominant to *r′* and *r*.

The gene i, a recessive intensifier. — In many crosses a kind of segregation has been encountered in which the type with the stronger anthocyanin colour behaves as a recessive. As pointed out by THOMPSON (1938), the genes of the *R*-series influence the pigmentation of leaf and involucre only, but this new recessive gene, which will be called *i*, shows its effect also in other parts of the plants. Anthocyanin is in *ii*-plants under most conditions present in all parts of the leaf, including the dorsal side of the midrib, in the stem and on the involucral bracts. The gene interacts with the genes *R*—*r″*—*r′* and *r* to give more intensely coloured variations of the different types. Plants of the genotype *RRii* assume a very deep red colour. Whereas the anthocyanin colour produced by the genes *R*, *r″* and *r′* is brownish red, the intensified pigmentation caused by the presence of *i* is of a violet shade. Although

TABLE 3. *F_2-data for crosses segregating I—i.*

No.	Cross parents	Parental genotypes	Number of fam.	Segregation *I*	Segregation *i*	$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$
1	R.Liebl.×V.grasse	*ii*×*II*	3	108	32	0.343	0.076
2	Laur.×Merv.	*II*×*ii*	2	80	27	0.003	0.076
3	Oakl.×Merv.	*II*×*ii*	3	171	44	2.358	0.300
4	Oakl.×Fl. 23	*II*×*ii*	1	124	35	0.757	—
5	Wond.×Cont.	*II*×*ii*	5	181	61	0.006	6.121
6	K.driv×Merv.	*II*×*ii*	1	195	61	0.188	0.002
7	Gr. 10×Laur.	*ii*×*II*	1	83	17	3.413	—
8	tttete×Fl. 23	*II*×*ii*	1	57	18	0.040	—
9	T+g×*IIGG*	*ii*×*II*	3	315	105	0.000	0.013
10	*iiGG*×*IIgg*	*ii*×*II*	3	206	61	0.660	2.802
11	Oakl.×Altaica	*II*×*ii*	3	164	62	0.714	1.369
12	K.driv×Altaica	*II*×*ii*	3	165	41	2.854	0.128
13	Berl.×Altaica	*II*×*ii*	3	159	49	0.231	0.184
14	R.rouge×Sc.M	*ii*×*II*	3	156	59	0.684	1.743
15	Sc.L×Altaica	*II*×*ii*	5	458	139	0.939	2.566
16	R.Liebl.×Sc.I	*ii*×*II*	1	37	23	5.689*	—
17	T+g×Rosette 48	*ii*×*II*	3	227	65	1.169	3.723
18	Berl.×LC	*II*×*ii*	4	261	86	0.009	0.135
			Total:	3147	985	2.974	
			$\chi^2_{\text{het.(17)}}$ for crosses = 17.083				
19	Sc.H×Fl. 23	*II*×*ii*	1	348	22	71.643***	—
20	Altaica×Sc.H	*ii*×*II*	5	414	38	66.372***	23.373***
21	Sc.H×T+g	*II*×*ii*	1	51	10	2.410	—
22	Sc.S.Afr.×Altaica	*II*×*ii*	3	227	27	27.974***	1.369
23	SL×Altaica	*II*×*ii*	3	173	36	6.738**	2.531
24	Asp.×Fl. 23	*II*×*ii*	1	184	45	3.495	—
			$\chi^2_{\text{het.(5)}}$ for crosses = 39.296***				

some variation is found in the manifestation of the gene, the recessive type is generally easily recognized in segregating families.

The data in Table 1 show for *I—i* a significant deviation from the expected 3 : 1 ratio and a significant heterogeneity between crosses. Data for the individual crosses have been presented in Table 3. When the results from the last six crosses in this table, no. 19—24, are omitted, the remaining crosses show good agreement with each other and the total is a reasonably good fit for 3 : 1. The last six crosses have been excluded from the total because the *II*-parent in each of them is a line which has been found to induce partial pollen sterility in F_1-hybrids

(LINDQVIST, 1960 a, p. 104). The association between partial pollen sterility in F_1 and abnormal segregation for *I—i* in F_2 will be discussed later in this paper. Meanwhile it should be noted that the six crosses do not form a homogeneous group, the χ^2 for heterogeneity being significant at the 0.1 % level.

F_2-data from the cross R. Liebl. × V. grasse, showed satisfactory agreement with expectation. Out of 18 progenies from *I*-plants, two bred true for the dominant character and 16 segregated, giving in total a ratio of 308 : 103, the χ^2 for heterogeneity between families being 16.255 (P=0.3 — 0.5). Five progenies from *ii*-plants bred true for the recessive character.

The recessive genes segregating in the crosses in Table 3, originate from five different sources, namely R.Liebl., Merv., Cont., R.rouge and Altaica. Crosses have been made between these forms in order to test the identity of the genes and in every case the F_1-hybrids were found to have the intensified anthocyanin pigmentation, which shows that all five lines are *ii*. This gene is also present in the winter cabbage lettuce variety Rousse d'hiver and in the primitive *L. sativa* form LC (both *CCTTrrii*) as shown by other crosses.

The gene v for vanishing anthocyanin colour. — The form of *L. serriola* originating from South Africa, Sc.S.Afr., has a characteristic kind of anthocyanin pigmentation. Young plants show the anthocyanin colour typical of the 'tinged' type, but as they grow older the pigment gradually disappears. When the plants reach the flowering stage, anthocyanin is present only on the dorsal side of the petals and in the prickles of stems and leaves. At this stage the plants appear at first sight completely lacking in anthocyanin. Other forms of *L. serriola* have anthocyanin colour on prickles and petals like Sc.S.Afr., but also on leaves, stems, flower head stalks and involucral bracts.

Sc.S.Afr. has been crossed with Sc.H, Altaica and with various cultivated forms. In all F_1-hybrids anthocyanin colour persisted throughout the development of the plant, but plants with fading pigmentation appeared in F_2. Segregating families were classified twice, once soon after being transplanted to the field and once at flowering stage. This was necessary especially in those crosses where the gene pair *T—t* also segregated and where therefore a proportion of the plants lacked anthocyanin from the beginning.

The F_2-data in Table 1 show a serious shortage of the recessive type and highly significant heterogeneity between crosses. F_2-results of the 7 crosses are presented individually in Table 4. Segregation is normal

TABLE 4. *Inheritance of the character 'vanishing anthocyanin'. F_2-data.*

Cross		Number of fam.	*V*	*v*	$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$
No.	Parents					
1	Sc.S.Afr.×Sc.H	3	398	116	1.621	0.142
2	Sc.S.Afr.×Altaica	3	196	59	0.472	2.215
3	Oakl.×Sc.S.Afr.	2	126	12	19.565***	0.136
4	Sc.S.Afr.×Oakl.	2	104	8	19.048***	0.048
5	Berl.×Sc.S.Afr.	6	315	48	26.851***	2.989
6	Sc.S.Afr.×Maik.	2	92	14	7.862**	0.517
7	Maik.×Sc.S.Afr.	6	482	81	33.819***	2.033

in two of the crosses, but disturbed in the other five, which give a total of 1119 *V* : 163 *vv* ($\chi^2_{3:1}=103.198$***; $\chi^2_{\text{het.(4)}}=1.214$). It has been shown in an earlier paper (LINDQVIST, 1958) that the recessive, *v*, is absolutely or extremely closely linked to the gene U_{lob}, which is responsible for the character lobed leaves. The segregation at the *U*-locus is often drastically disturbed, particularly in crosses between *L. serriola* and *L. sativa*, and there can be no doubt that the influences which cause these disturbances are also the cause of the abnormal segregation ratios of the gene pair *V*—*v*. We will return to this question later in this paper.

The gene pair *V*—*v* segregated also in the cross R.rouge×Sc.S.Afr., but the F_2-generation was not classified for anthocyanin characters. The segregation for lobed versus non-lobed leaves was normal in this cross (*cf.* LINDQVIST, 1958, Table 5) and there is thus every reason to believe that the segregation for *V* and *v* was normal also. The progenies of 46 F_2-plants were tested in F_3. Six were found to breed true for the dominant character, 23 segregated, giving a total of 566 *V* and 204 *vv* ($\chi^2_{3:1}=0.916$; $\chi^2_{\text{het.(22)}}=22.032$) and 17 bred true for the recessive character. Apart from the excess of *vv*-families which is due to an intentional selection of lobed plants, these results agree well with expectation on the basis of monohybrid inheritance.

Interaction between v and other anthocyanin genes. — As was mentioned above, anthocyanin pigmentation on the dorsal side of the petals and in prickles is not suppressed by *v*. Absence of anthocyanin in the surrounding tissue makes the prickles very conspicuous in *vv*-plants.

Spots and coloured areas on leaves and involucral bracts, produced by the alleles *R*, *r''* and *r'* are not influenced by *v*. In plants with the

allele *R* the strong pigmentation, present on the involucre after flowering, stands out very sharply against a background devoid of anthocyanin and gives the plants a very characteristic appearance.

The intensified pigmentation of plants homozygous for the recessive gene, *i*, disappears in plants homozygous for *v*. It does not show itself to the full extent in the heterozygote, *Vv*, and can also in such plants be completely suppressed in the presence of other genes. This interaction is well demonstrated by an F_3-family from the cross R.rouge×Sc.S.Afr. The parent plant of this family had lobed leaves, but as mentioned above, the F_2-generation was not classified for anthocyanin colour. The F_3-family in question segregated in the following manner:

$U_{lob}U_{lob}vv$		$U_{lob}uVv$		$uuVV$		Total
I	*ii*	*I*	*ii*	*I*	*ii*	
10	0	14	5	0	10	39

Because of the absolute linkage between U_{lob} and *v*, lobed plants with the *v*-character must be homozygous $U_{lob}U_{lob}$ and lobed plants with the normal anthocyanin colour must be heterozygous at both loci.

The family contained 15 plants with the intensive pigmentation typical for *ii*-plants and these 15 plants included all the 10 non-lobed plants, a fact which at first sight suggested linkage between *U* and *I*. The association between non-lobed leaves and intensive pigmentation in this family was, however, without doubt due to interaction between *v* and *i*. The parent plant was apparently heterozygous for U_{lob} and *V*, but homozygous for the recessive *i*. In the F_3-family this recessive gene showed its effect in the 10 non-lobed plants, as they were homozygous for *V*, but only in 5 of the 19 plants heterozygous for *V*. This fact suggests, that another gene, or other genes, segregated in this family which are capable of suppressing the effect of *i*, when *v* is present in single dose.

This interaction must, of course, have the effect of creating a deficiency in the *ii*-class in F_2 of crosses where *I* and *V* segregate together, but it cannot have played any important part in connection with the abnormal segregation ratios for *I*—*i* in Table 3, as the genes *V*—*v* segregate only in one cross, Altaica×Sc.S.Afr.

2. Leaf colour

By leaf colour is here understood the different shades of green and yellow imparted by the plastids, and not the anthocyanin pigmentation.

TABLE 5. *Inheritance of leaf colour; F_2-data from crosses between light green and yellow.*

Parents	Leaf colour of parents	Number of fam.	Light green	Yellow	Total	$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$
Berl.×Oakl.	yellow×light	7	512	148	660	2.335	4.181
Berl.×K.driv	„ „	6	498	148	646	1.505	7.896
Berl.×Montrée	„ „	5	274	107	381	1.933	3.045
Montrée×Berl.	light×yellow	1	89	32	121	0.135	—
Oakl.×Fl. 23	„ „	4	300	91	391	0.622	0.074
K.driv×Fl. 23	„ „	1	214	78	292	0.457	—
Tezier×Fl. 23	„ „	1	71	23	94	0.014	—
		Total	1958	627	2585	0.765	

$\chi^2_{\text{het.(6)}}$ for crosses = 6.236

Inheritance of leaf colour in lettuce has been studied by BREMER (1931) and THOMPSON (1938). BREMER (*l. c.*) found that the golden yellow colour of the variety Rudolph's Liebling behaved as a simple recessive character. BREMER did not give this gene a name, but ERNST-SCHWARZENBACH (1936) assigned to it the letter *g*. Without any reference to BREMER's work, THOMPSON (*l. c.*) used the same letter for a recessive gene responsible for the light green colour of the variety Hanson. There is, however, a distinct difference between the light green colour of the variety Hanson and the golden yellow colour of varieties like Rudolph's Liebling and Berliner, a fact which raises the question whether the two genes are identical.

Evidence for the existence of two loci for leaf colour. — The variety Hanson was not available when this work was carried out, but comparisons made later have shown that it has a leaf colour very similar to that of the lines Oakl., K.driv, Montrée and Tezier. These four lines have been crossed with two lines with golden yellow leaf colour, Berl. and Fl. 23 (from the cross R.Liebl.×Sc. I). The leaf colour of the F_1-hybrids was similar to that of the light green parent and there was a clear 3 : 1 segregation in F_2 as shown by the data in Table 5. An F_3-generation of the cross Berl.×Montrée was classified for leaf colour with the following results:

Number of families		Total of segregating families		
True-breeding	Segregating	Light green	Yellow	$\chi^2_{3:1}$
22	37	1947	635	0.703

$\chi^2_{\text{het. (36)}}$ for families 23.478

The agreement with expectation is very good, both with regard to the proportion between true breeding and segregating families ($\chi^2_{1:2}=1.350$; $P=0.2-0.3$) and the ratios found in the segregating families. These results leave no doubt that different genes are responsible for the light green and the yellow colours. There was a certain amount of variation within the light green class in these crosses and darker green plants were observed, but their numbers were not recorded.

The fact that the three combinations green×light green, green× yellow and light green×yellow all give monohybrid ratios in F_2, suggests that the genes for light green colour and for yellow colour may be alleles of the same locus. Both genes are linked to the gene *H*, a gene for hearting. This is proved by the data in Table 18, where results from crosses segregating for *H—h* and for green versus yellow leaf colour are found under the heading *G—H* and crosses segregating for *H—h* and for green versus light green colour under the heading *H—Lg*. The data in Table 18 also show, however, that the genes for yellow and light green colour differ with regard to the strength of the linkage with *H*. Combining the data for the two genes and testing for agreement in crossing over values, using the scoring method, gives a heterogeneity χ^2 of 31.671***, which leaves no doubt about the significance of the difference.

On the basis of these results it must be concluded that the genes for light green leaf colour and for yellow leaf colour are non-allelic, but that they are situated on the same choromosome. The letter *g* will be retained for the gene producing yellow colour and the gene for light green colour will be designated as *lg*. It is difficult to explain why crosses between light green and yellow do not produce pure green in F_1, as is to be expected if the genes are non-allelic. It is possible that plants heterozygous at both loci are incapable of producing the green colour. The occurrence in F_2 of plants with darker leaves can be accounted for by crossing over between the two loci, but this question must be investigated further.

Disturbed segregation ratios for Lg and G. — The summarized data in Table 1 show both for *Lg* and *G* poor agreement between crosses, and also a serious deficiency in the recessive class. Results of individual crosses presented in Table 6 show that segregation is normal in some crosses, but drastically disturbed in others. There is serious heterogeneity also between the crosses with disturbed segregation ratios.

In Table 6 figures for the pollen fertility of the F_1-generations have been entered and it is clear that reduced pollen fertility and disturbed

TABLE 6. *Inheritance of leaf colour; F_2-results of individual crosses.*

No.	Cross	Leaf colour of parents	Number of fam.	Segregation			$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$	Fertility in F_1	
				G	*g*	Total			♂	♀
1	Berl.×Gr. 10	Yellow×green	3	300	89	389	0.933	0.780	—	—
2	Berl.×Wond.	,, ,,	2	99	27	126	0.857	0.719	98.8	88.9
3	Berl.×Altaica	,, ,,	6	337	122	459	0.611	7.998	100.0	—
4	Berl.×LC	,, ,,	4	360	107	467	1.086	2.614	97.4	91.9
5	Berl.×Sc.R	,, ,,	2	190	52	242	1.592	0.328	95.4	80.1
6	Berl.×Sc.L	,, ,,	3	241	70	311	1.030	2.228	94.8	96.2
7	R.Liebl.×V.grasse	,, ,,	3	169	46	215	1.490	1.314	—	—
8	R.Liebl.×Sc.I	,, ,,	9	508	162	670	0.241	8.478	—	—
9	T+g×Rosette 48	,, ,,	3	226	66	292	0.895	3.322	96.8	—
10	T+g×IIGG	,, ,,	3	304	116	420	1.537	0.230	—	—
11	Fl. 23×Altaica	,, ,,	3	231	60	291	2.979	2.142	—	—
		Total		2965	917	3882	3.932*			
				$\chi^2_{\text{het.(10)}}$ for crosses = 9.319						
12	Berl.×Asp.	Yellow×green	6	471	110	581	11.406***	5.593	86.3	90.3
13	Berl.×SP	,, ,,	2	209	51	260	4.021*	0.877	80.4	—
14	Berl.×SL	,, ,,	6	416	83	499	18.630***	8.351	45.5	—
15	Berl.×Sc.S.Afr.	,, ,,	5	340	42	382	39.962***	8.583	67.4	92.5
16	Berl.×Sc.H	,, ,,	12	783	84	867	108.405***	20.081*	67.5	—
17	Sc.H×Fl. 23	Green×yellow	1	337	37	374	45.522***	—	—	—
18	Asp.×Fl. 23	,, ,,	1	264	44	308	18.857***	—	—	—
				$\chi^2_{\text{het.(8)}}$ for crosses = 43.367***						
19	K.driv×Altaica	Light green×green	3	143	35	178	2.704	0.601	98.9	—

					l_g	l_g				
19	K.driv×Altaica	Light green×green	3	143	35	178	2.704	0.601	98.9	—
20	K.driv×Gr. 10	„ „ „	3	226	74	300	0.018	0.258	—	—
21	Tezier×R.verte	„ „ „	3	240	62	302	3.219	4.081	—	—
22	Maik.×Altaica	„ „ „	2	156	60	216	0.889	0.180	97.4	95.6
23	Maik.×Rosette 48	„ „ „	3	226	56	282	3.976*	0.435	96.2	89.4
		Total		991	287	1278	4.408*			
				$\chi^2_{het.(4)}$ for crosses = 6.398						
24	Oakl.×Sc.S.Afr.	Light green×green	2	151	18	169	18.558***	0.206	53.7	85.7
25	Maik.×Sc.S.Afr.	„ „ „	6	497	69	566	49.529***	15.920**	46.4	74.4
26	Oakl.×Asp.	„ „ „	2	168	46	214	1.402	1.751	86.5	84.0
27	SB×Maik.	Green×light green	2	226	54	280	4.876*	0.723	58.6	—
28	Sc.S.Afr.×Oakl.	„ „ „	2	126	8	134	25.881***	0.960	49.4	—
29	Sc.S.Afr.×Maik.	„ „ „	2	96	10	106	13.698***	—	42.5	—
30	Asp.×Oakl.	„ „ „	1	59	14	73	1.320	—	—	—
				$\chi^2_{het.(6)}$ for crosses = 29.831***						

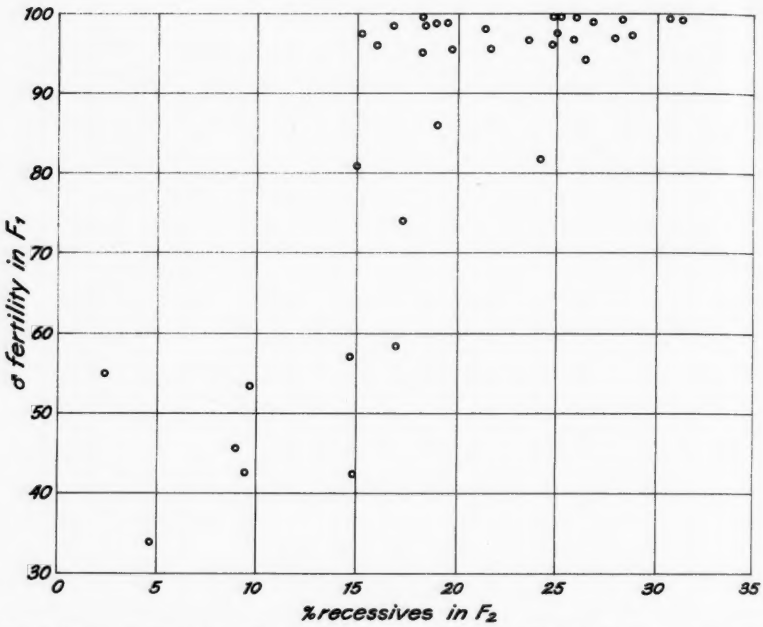


Fig. 1. Diagram illustrating the relation between pollen fertility in F_1 and segregation for leaf colour in F_2.

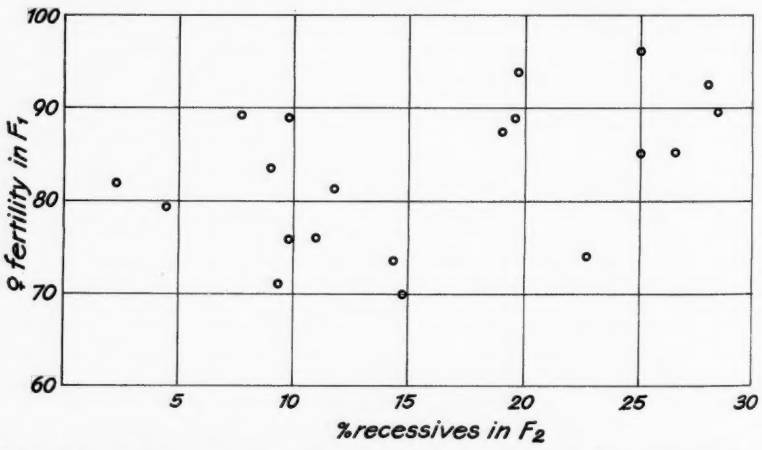


Fig. 2. Diagram illustrating the relation between seed setting in F_1 and segregation for leaf colour in F_2.

segregation go together. The pollen fertility data in Table 6 do not always refer to those F_1-plants from which the F_2-families originate, but are averages of all available data for each F_1-combination. Pollen fertility data for 37 F_1-plants, which are parents of F_2-families in which leaf colour segregation has been recorded, have in Fig. 1. been plotted against percentage of recessives in the F_2-families and it is obvious that there is a strong correlation. The coefficient of correlation, 0.774, is highly significant (t for 35 DF=7.226; $P < 0.001$).

The presence of partial pollen sterility in hybrids within the *L. sativa-serriola* ecospecies has earlier been established by the writer (LINDQVIST, 1960 a). It was found that this sterility occurred only in crosses in which one of the following seven lines took part: Asp., SL, SB, SP, Sc.H, Sc.S.Afr. and Sc.J. The data in Table 6 show that the first six of these lines are involved in 12 out of the 13 crosses with disturbed segregation for leaf colour. There is therefore every reason to believe that there is a causal connection between pollen sterility and disturbed segregation of the leaf colour genes.

The segregation ratios for leaf colour appear to be correlated also with seed setting although less strongly than with pollen fertility. This can be seen from the data in Table 6 and the diagram in Fig. 2, which illustrates the relation between seed setting in 20 F_1-plants and percentage recessives in their offspring. The correlation coefficient, 0.433, is barely significant at the 5 % level (t=2.038 for 18 DF; P=0.05—0.10).

F_2-generations were raised of the three crosses Berl.×Sc.R (no. 5 in Table 6) and Berl.×Sc.H (no. 16) and Sc.H×Fl. 23 (no. 17). The following numbers of true-breeding and segregating families were obtained from green F_2-plants:

Crosses	Number of families		χ^2
	True-breeding	Segregating	
(5) Berl.×Sc.R			
Observed	13	29	
Expected, 1 : 2	14.0	28.0	0.107
(16) Berl.×Sc.H			
Observed	20	22	
Expected, 1 : 2	14.0	28.0	3.857*
„ on F_2 segr.	18.7	23.3	0.163
(17) Sc.H×Fl. 23			
Observed	13	10	
Expected, 1 : 2	7.7	15.3	5.484*
„ on F_2 segr.	10.2	12.8	1.281

The ratio between true-breeding and segregating families in Berl.× Sc.R is in good agreement with the expected 1 : 2 ratio and with the normal segregation found in F_2 of this cross, but in the other two crosses there is a large excess of true-breeding families as is to be expected if the deficiency of recessives in F_2 is due to a deficiency of functioning gametes with the gene *g*. The expected frequencies of the two types of family have been calculated on the basis of the ratios observed in F_2 on the assumption that the female gametes function normally whether they have *G* or *g*, and the agreement between observed and expected frequencies was found to be good. Some of the families were rather small, the number of plants varying between 18 and 67. However, even in the smallest families the recessive type is expected to appear in 84 cases out of 100 if the segregation is the same as found in F_2. It is therefore unlikely that the observed number of true-breeding families is seriously inflated because of the small size of the F_3-progenies.

Results of segregating families from the three crosses are presented in Table 7. The total of the 29 families from the cross Berl.×Sc.R shows a slight deficiency in the recessive class, but the homogeneity of the data is good. The deficiency of yellow leaved plants in these families and in F_2 of the first 11 crosses in Table 6, is presumably due to a slightly reduced viability.

The 10 segregating families from the cross Sc.H×Fl. 23 all behaved in the same manner as the F_2-generation, *i. e.* there was a serious deficiency in the recessive class. The χ^2 for heterogeneity between the families was 8.102 which corresponds to a P-value of 0.5—0.7. The total of all the families was 422 green : 33 yellow, which agrees reasonably well with the result of F_2, 337 green : 37 yellow ($\chi^2_{het.}=1.851$; P=0.10—0.20).

In the F_3-generation of Berl.×Sc.H different segregation ratios were, however, found in different families. The heterogeneity χ^2 was found to be 59.047, and the corresponding P-value is <0.001. Some families had a significant deficiency in the recessive class, others showed a normal 3 : 1 segregation, but no family had a significant excess in the recessive class. The fact that some F_3-families segregate normally, indicates that the disturbed F_2-ratios are caused, not by the gene *g* itself but by a pollen lethality factor which is linked with the *G* locus. As the parent lines have normal pollen fertility, the partial sterility observed in F_1 and the abnormal ratios in F_2 must be due to a complementary effect of two or more such factors. A discussion of this question follows later in this paper.

TABLE 7. *Inheritance of leaf colour; F_2-data from crosses segregating for G—g.*

Berl.×Sc.R				
Fam. no.	*G*	*g*	Total	$\chi^2_{3:1}$
30654	26	15	41	2.935
55	45	10	55	1.364
58	47	12	59	0.684
59	36	14	50	0.240
60	42	11	53	0.509
61	39	16	55	0.491
64	48	9	57	2.579
65	48	9	57	2.579
69	49	9	58	2.782
70	46	7	53	3.931
71	46	11	57	0.988
73	43	14	57	0.006
75	37	11	48	0.111
77	42	13	55	0.055
78	43	11	54	0.617
81	43	11	54	0.617
82	37	13	50	0.027
83	35	10	45	0.185
84	34	14	48	0.444
86	32	10	42	0.032
87	36	13	49	0.061
88	43	6	49	4.252*
89	38	13	51	0.007
92	26	11	37	0.441
93	35	13	48	0.111
96	37	16	53	0.761
97	44	10	54	1.210
98	33	12	45	0.067
30700	38	13	51	0.007
Total	1148	337	1485	4.213*
				$\chi^2_{het.(28)}=23.880$
				P=0.50—0.70

Sc. H×Fl. 23				
Fam. no.	*G*	*g*	Total	$\chi^2_{3:1}$
30241	41	2	43	9.496**
42	42	7	49	3.000
44	44	3	47	8.688**
46	46	1	47	13.113***
48	46	2	48	11.111***
51	38	3	41	6.837**
53	41	4	45	6.230*
56	45	3	48	9.000**
59	38	5	43	4.101*
62	41	3	44	7.758**
Total	422	33	455	76.432***
				$\chi^2_{het.(9)}=8.102$

Berl.×Sc. H				
Fam. no.	*G*	*g*	Total	$\chi^2_{3:1}$
30554	34	5	39	3.085
58	32	9	41	0.203
60	48	2	50	11.760***
62	48	19	67	0.403
65	16	2	18	1.852
66	46	1	47	13.113***
68	15	8	23	1.174
71	22	7	29	0.011
75	27	5	32	1.500
77	28	5	33	1.707
80	28	3	31	3.882*
81	26	2	28	4.762*
82	39	7	46	2.348
87	20	3	23	1.754
91	20	12	32	2.667
92	27	4	31	2.419
93	46	3	49	9.313**
95	18	7	25	0.120
96	14	4	18	0.074
98	31	15	46	1.420
30600	17	4	21	0.397
04	38	2	40	8.533**
Total	640	129	769	27.476***
				$\chi^2_{het.(21)}=59.047***$

Spontaneous mutation G→g. — A single plant progeny from the green leaved, long-day variety Cheshunt Early Giant was found to consist of 22 green leaved and 5 yellow leaved plants, all of the long-day type. There was no segregation for any other characters, and the yellow leaved plants agreed with the green leaved in everything except leaf colour. The variety Cheshunt Early Giant had previously been propagated for three generations and a total of 83 plants had been examined, all green leaved. Offspring from three green leaved plants in the segregating progeny gave one constant green family and two which segregated, giving a total of 47 green and 9 yellow. The total of all three segregating progenies was thus 69 green and 14 yellow, a ratio which agrees reasonable well with 3 : 1 (χ^2=2.943; P=0.05—0.10). The yellow leaved plants must owe their origin either to a spontaneous cross between Cheshunt Early Giant and a yellow leaved long-day plant or to a mutation. Since no yellow leaved long-day plants had previously existed in the experimental material, only the latter alternative remains. Yellow leaved plants from the original segregating progeny have been propagated for eight generations and have always bred true for the yellow colour. A cross has been made with a yellow leaved segregant from the cross R.Liebl.×V.grasse, which had inherited the recessive gene *g* from R.Liebl. The F_1 and F_2-generations of this cross consisted entirely of yellow leaved plants, which proves that the new type also has the recessive gene *g*. Thus there can be no doubt that a mutation *G→g* has occurred in Cheshunt Early Giant.

3. Lobing

Results presented by the writer in a previous paper (LINDQVIST, 1958) proved that lobing in lettuce is controlled by three alleles, U_{oak}, U_{lob} and *u*, and that the disturbed segregation ratios, which are often found for this character, are due to some abnormality at gametogenesis or at fertilization. Further data with a bearing on this question will be presented here.

Backcrosses. — The cross between Berl. and Sc.L was found to give a highly significant excess of non-lobed plants in F_2 (LINDQVIST, 1958, Table 5). Backcrosses between F_1 (Berl.×Sc.L) and non-lobed plants have been made in both directions. Three crosses with the F_1 as male partner gave the following results:

Crosses	Lobed	Non-lobed	Total	$\chi^2_{1:1}$
Laur. × F_1 (Berl. × Sc.L)	34	130	164	56.195***
Sc.H × „	47	145	192	50.021***
MS × „	14	78	92	44.522***
	Total 95	353	448	148.581***

$\chi^2_{\text{het. (2) for crosses}} = 3.228$
$\chi^2_{\text{het. (12) for fam.}} = 5.524$

A difficulty with backcrosses in lettuce is that it is practically impossible to avoid a certain proportion of selfs. It is therefore necessary to choose the parents in such a way that plants which have arisen through selfing can be identified and eliminated. In the first of the three crosses a seed colour gene was used as marker, Laur. having the recessive gene, *y*, for yellow seed colour, Berl. and Sc. L both having the dominant allele *Y* which gives brown seed colour. In the second cross there were no suitable marker genes, but various characters, such as flowering time and size of flowers and seeds, were used, and plants of the Sc.H line were interplanted for comparison. The mother of the third cross, MS, was a non-lobed male sterile (see below page 450) and selfing was therefore out of question in this case.

Two crosses were made with the F_1 as female partner:

Crosses	Lobed	Non-lobed	Total	$\chi^2_{1:1}$
F_1 (Berl. × Sc.L) × Laur.	71	56	127	1.772
„ × Sc.H	28	41	69	2.449
	Total 99	97	196	0.020

$\chi^2_{\text{het. for crosses}} = 4.201^*$
$\chi^2_{\text{het. (4) for fam.}} = 0.719$

The gene *r'* for spotted leaves, which is present in Laur., served as marker gene in the first cross, both Berl. and Sc.L having the recessive allele *r*. For the cross F_1 (Berl. × Sc.L) × Sc.H the same applies as was said above about the reciprocal backcross. The total of the two crosses shows very good agreement with a 1 : 1 ratio. The χ^2 for heterogeneity is significant at the 5 % level, which presumably is due to difficulties in identifying selfs in the backcross F_1 × Sc.H.

The different results obtained when the F_1-hybrid is used as male and female parent, clearly show that the abnormal F_2-ratios in the cross Berl. × Sc.L are due to a difference in fertilizing efficiency of pollen grains carrying the genes U_{lob} and *u*. Meiosis is quite normal in this hybrid and also pollen fertility and seed setting, as shown by data presented in an earlier paper (LINDQVIST, 1960 a, Tables 12, 13 and 15). Pollen grains carrying the allele U_{lob} are thus normal in appearance, but

it has not been possible to decide whether they are capable of germination.

Data published earlier on the segregation of lobing (LINDQVIST, 1958) and on pollen fertility (LINDQVIST, 1960 a) in various crosses within *L. sativa—serriola*, confirm that there is no connection between pollen sterility and aberrent F_2-ratios for lobing. As examples it may be mentioned that the four crosses Maik.×Sc.S.Afr., Berl.×Sc.S.Afr., Berl.×Sc.L and Berl.×Sc.R all gave a large excess of non-lobed plants in F_2, although the first two were partly pollen sterile and the last two had normal pollen fertility. Further the two crosses Sc.H×Sc.R and SL×Sc.L both gave normal 3 : 1 segregations although the first had normal pollen fertility and the second was partly pollen sterile.

An F_2-generation has been raised of the cross Sc.H×Sc.L and it gave a normal monohybrid ratio (total of 2 families 110 : 33; $\chi^2_{3:1}=0.282$; $\chi^2_{het.}=0.404$) whereas the cross Berl.×Sc.L gave a large excess of non-lobed plants as has been mentioned above. One possible explanation of the difference between these two crosses is that the styles of the F_1-hybrid Berl.×Sc.L has a retarding effect on U_{lob}-gametes, but that both kinds of gametes grow at the same rate in the styles of the hybrid Sc.H×Sc.L. If this is the case one would have reason to except a normal 1 : 1 segregation in the backcross Sc.H×F_1(Berl.×Sc.L), but as this backcross gave a large excess of the recessive, it must be concluded that there is no influence of the style, but that the difference in fertilizing efficiency is determined entirely by the genotype of the pollen grains.

The agreement is good between F_2 of the cross Berl.×Sc.L and the backcrosses in which F_1 was the male partner. The F_2 gave 179 lobed : 128 non-lobed. The total ratio of all backcrosses with F_1 as pollen parent, *i.e.* 95 lobed and 353 non-lobed, has been used for comparison, and the χ^2 for heterogeneity, calculated by the maximum likelihood method, was found to be only 0.595 (P=0.3—0.5).

F_3-data. — In the earlier paper on the inheritance of lobing (LINDQVIST, 1958) F_3-data were presented in a summarized form and it was shown that in crosses with disturbed F_2-ratios there was a significant heterogeneity between F_3-families in most of the crosses. A study of the individual families from such crosses shows, that they can be divided into three groups: (1) families segregating 3 : 1, (2) families having an excess in the recessive class and (3) families having a deficiency in the recessive class. It appears unnecessary to publish the result of each family, but the number of families in each group is found in Table 8

TABLE 8. *Inheritance of lobing; F_s-data.*

Classification of families				Segregation data for families from K. driv×Fl. 23								
Crosses	Number of families segregating			3:1			Excess recessives			Deficiency recessives		
	3:1	excess recess.	def. recess.	U_{lob}	u	$\chi^2_{3:1}$	U_{lob}	u	$\chi^2_{3:1}$	U_{lob}	u	$\chi^2_{3:1}$
R.Liebl.×Sc.I	7	6	0	73	25	0.014	48	46	28.723***	92	5	20.375***
				76	18	1.716	33	47	48.600***			
K.driv×Fl. 23	14	8	1	49	25	3.045	41	57	57.482***			
				63	30	2.613	45	42	25.138***			
Berl.×Fl. 23	5	17	1	75	17	2.087	62	33	4.804*			
				75	23	0.122	45	48	35.129***			
Berl.×Sc.R	17	9	0	68	29	1.241	48	34	11.854***			
				71	25	0.056	54	43	19.330***			
K.driv×Eichenbl.	13	6	1	70	29	0.973						
				69	17	1.256						
Oakl.×K.driv	9	6	0	45	10	1.364						
				71	28	0.569						
Oakl.×Forcing	21	1	3	65	32	3.302						
				17	7	0.222						
TTtete×Oakl.	4	—	4									

for eight crosses which gave abnormal F_2-ratios. Results of the individual families of the cross K.driv×Fl. 23 are also shown, as an example.

The families with a deficiency in the recessive class are probably underrepresented because the number of plants per family was often too small to allow the recessive type to appear when the expected proportion was low. The number of plants per family is also insufficient to distinguish in every case between families segregating 3 : 1, and those with an excess in the recessive class. For this purpose the line has been drawn at a deviation from 3 : 1, significant at the 5 % level. The classification of the families in Table 8 is therefore somewhat unreliable, but the fact that families with normal monohybrid segregation appear in F_3, proves that male gametes with different alleles at the *U*-locus do not always differ in fertilizing efficiency. The abnormal segregation ratios for lobing must therefore be due to one or more genes which are linked to the *U*-locus, *i. e.* gametophyte factors such as have been found in maize (MANGELSDORF and JONES, 1926) and in many other plant species. Until the opposite has been proved it will be assumed that only one gametophyte locus is involved and the allele with a detrimental effect on the pollen will be designated as *ga*. F_3-families with normal monohybrid segregation owe their origin to one cross-over gamete and one gamete of the parental type, and those showing a deviation opposite to that of F_2 owe their origin to two cross-over gametes.

The size of the deviation from the normal monohybrid ratio in segregating families, depends partly on the proportion of *Ga* and *ga* gametes which accomplish fertilization, and partly on the amount of crossing over between *Ga* and *U*. The crossing over value for *Ga* and *U* can be calculated only, if it is assumed that gametes carrying *ga* are completely ineffective in accomplishing fertilisation (*cf.* BEMIS, 1959). This is apparent from the fact that the maximum likelihood method leads to the following formula for the calculation of the crossing over value from such F_2-data (repulsion phase) : $p=\frac{a_1-a_2-y(a_1+a_2)}{(a_1+a_2)(1-2y)}$, where p is the crossing over value, y the proportion of functioning *ga* gametes and a_1 and a_2 the observed frequencies in the dominant and recessive class respectively. The inheritance of lobing in lettuce is very similar to that of defective seeds in maize, which was analyzed by MANGELSDORF and JONES (1926). They pointed out that additional information is necessary for the estimation of y and p from the F_2-data and they were able to obtain such information because another gene, *Su*, was linked

with *de*, the gene for defective seeds. In lettuce no other gene is known on the same chromosome as *U* and *Ga*. It is, however, theoretically possible to estimate p with the aid of the F_3-data, as the proportion of F_3-families showing different kinds of segregation is independent of y. The maximum likelihood method gives the following formula:

$$p = \frac{a_1 + 2a_3}{2(a_1 + a_2 + a_3)} \pm \sqrt{\frac{p(1-p)}{2n}}$$

where a_1, a_2 and a_3 stand for the number of families of the three kinds, a_1 those segregating 3 : 1, a_2 those segregating like F_2 and a_3 those with a deviation opposite to F_2. The difficulty with this method is that a large number of plants is required in each F_3-family in order to make possible an accurate classification into the three categories. The results in Table 8 give for p an estimate of 32.07 ± 2.668 %. The agreement is, however, very poor between the number of families of each kind, expected at this value for p, and the numbers actually observed, and the reason is, without doubt, that many families with a deficiency in the recessive class have been classified as non-segregating, because of the small number of plants per family. Accurate determination of p and y must therefore wait until data are available from F_3-generations with larger families. It is, however, clear from the existing data that the linkage is rather loose and that the effect of *ga* is strong.

The F_2-data presented in the earlier paper on inheritance of lobing (LINDQVIST, 1958) cannot be explained purely by the existence of one gametophyte locus with two alleles. This is obvious if for example, the results of the five crosses Oakl. × Fl. 23, K.driv × Fl. 23, Berl. × Fl. 23, Oakl. × K.driv and Berl. × Oakl. are considered (LINDQVIST *l. c.*, Tables 3—5). The first cross gave a highly significant excess of oakleaved plants, the second and third an excess of non-lobed plants. This suggests that Oakl. has the genes $U_{oak}Ga$, Fl. 23 the genes $U_{lob}ga$ and Berl. and K.driv *u Ga*. In agreement with this assumption the cross Berl. × Oakl. gave a 3 : 1 ratio. The cross Oakl. × K.driv, however, gave a highly significant excess in the recessive class which cannot be explained on this hypothesis. Whether it is due to the existence of more than one allele at the *Ga*-locus or to the presence of more than one gametophyte locus, cannot be decided at present. Another problem which remains to be solved is the frequent occurrence of heterogeneity between families of the same cross (LINDQVIST, 1958).

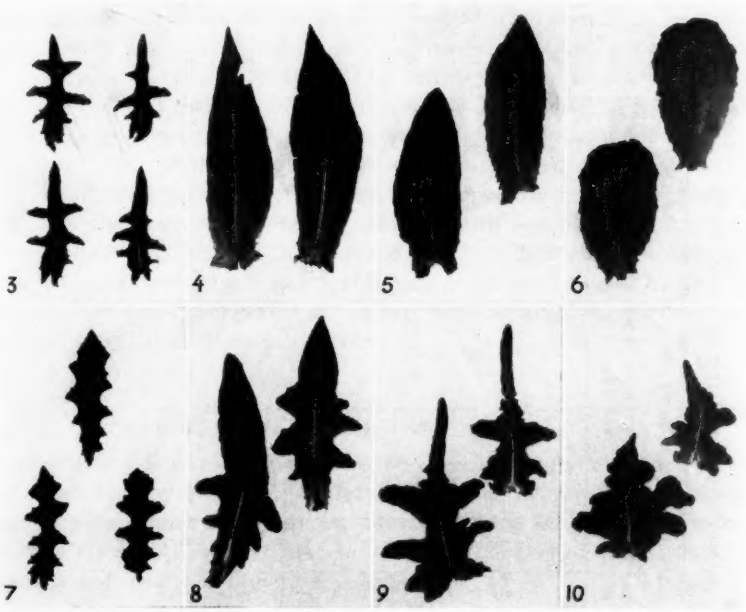
4. Shape of leaf apex

Variation. — Cultivated lettuce varieties have as a rule, leaves with rounded apex, but some forms of asparagus and cos lettuce, *e. g.* Asparagus Cos and Dear Tongue, have a clearly pointed apex. Among the material used in this study, the line Asp. and the primitive forms SL, SP and LC have long, narrow leaves with pointed apex. Altaica has shorter, less definitely pointed leaves, but the rest, including all lines of *L. serriola*, have blunt or rounded leaf apex. Pointed leaves are illustrated in Figs. 3 and 4 (Altaica and Asp.) and blunt leaves in Figs. 6 and 7 (R.rouge and *L. serriola*).

Inheritance. — The inheritance of leaf apex shape has been studied in 17 crosses. The pointed apex was found to be incompletely dominant. Figs. 2—4 illustrate the leaf apex shape of the lines Asp. and R.rouge and their F_1-hybrid. Monohybrid segregations were recorded in F_2. The genes for pointed and blunt apex have been designated as *P* and *p* respectively. Classification was usually easy and the data in Table 1 show good agreement with expectation and no heterogeneity between crosses or families. An F_2-generation of the cross Asp.×Laib., gave true-breeding and segregating families in the ratio 5 : 15, and the 15 segregating families gave a total of 374 pointed to 146 blunt ($\chi^2_{3:1}=2.626$; $\chi^2_{het.(14)}=16.258$). An F_2-generation of the cross Sc.L×Altaica also gave 5 true-breeding and 15 segregating families. Only three of the segregating families were fully classified giving a total of 68 pointed and 24 blunt ($\chi^2_{3:1}=0.058$; $\chi^2_{het.(2)}=2.483$).

In order to find out whether a different allele at the *P*-locus is responsible for the semi-pointed apex of Altaica, crosses were made between this line and Asp. and SL, but the results were inconclusive. A continuous variation was observed in F_2, but as no F_3-data are available it is not possible to decide whether this was due to segregation at the P-locus or segregation of other genes influencing the shape of the lamina.

Crosses segregating for pointed versus blunt leaves and, at same time, for lobed or oakleaved versus non-lobed, provided an opportunity of studying the interaction between the leaf form characters and the shape of the apex. It was observed that in lobed and oakleaved plants with pointed apex the incisions were confined to the lower and middle part of the leaf, the apical part being non-lobed and gradually tapering to a point. On lobed or oakleaved plants with blunt apex the part beyond the last incision came much more abduptly to an end. This difference is illustrated by Figs. 7—10.



Figs. 3—10. Variation in shape of leaf apex and interaction between genes for lobing and genes for shape of leaf apex. — Fig. 3. Altaica; $U_{lob}U_{lob}PP$. Fig. 4. Asp.; *uu PP*. Fig. 5. F_1Asp.×R.rouge; *uu Pp*. Fig. 6. R.rouge; *uu pp*. Fig. 7. F_1Sc.H×Sc.L; $U_{lob}u$ *pp*. Fig. 8. F_1Asp.×Altaica; $U_{lob}u$ *PP*. Fig. 9. F_1Asp.×Oakl.; $U_{oak}u$ *Pp*. Fig. 10. Oakl.; $U_{oak}U_{oak}$ *pp*.

In many crosses a correlation was observed between the shape of the apex and the shape of the lamina, pointed apex usually being associated with long, narrow leaves and vice versa. This is clearly demonstrated by Table 9, which shows the distribuition of plants with different length/width indices among F_2-plants with pointed and blunt apex. The length/width indices are based on measurements of the length and greatest width of three basal leaves from each plant. Twenty F_3-plants of the cross Asp.×Laib. could, with the aid of F_3-results be classified into homozygotes and heterozygotes. This cross therefore provides an opportunity of comparing heterozygotes with the two kinds of homozygotes. The average values for length/width index were for *PP*-plants 2.63, for *Pp*-plants 1.86 and for *pp*-plants 1.43 (see Table 9). The difference between homozygous pointed and heterozygotes is not statistically significant ($d=0.77\pm0.501$; $P=0.1$—0.2), but the results indicate

TABLE 9. *Correlation between shape of leaf apex and length/width index.*

Parent lines or crosses	Apex	Number of plants in different length/width index classes												Total number of plants	Average l/w index
		0.70-0.99	1.00-1.29	1.30-1.59	1.60-1.89	1.90-2.19	2.20-2.49	2.50-2.79	2.80-3.09	3.10-3.39	3.40-3.69	3.70-3.99	4.00-4.29	4.30-4.59	
Asp.	pointed											1	2		3	4.04
Laib.	blunt	10	13												23	1.02
R.rouge	„			1	2	7	9	1							20	2.14
F_1Asp.×Laib.	pointed		1	5	5	1									12	1.55
F_1Asp.×R.rouge	„						2	5	2						9	2.70
F_2Asp.×Laib.	pointed	1	12	33	58	48	37	19	15	4	2	2	1		232	2.05
„ „	blunt	10	27	24	18	9	1								89	1.43
„ „	PP*				1	1	0	1	1	0	1				5	2.63
„ „	Pp**		3	2	5	2	2	0	1						15	1.86
F_2Asp.×R.rouge	pointed					4	21	21	16	16	4	4	2	1	89	2.86
„ „	blunt		1	0	5	13	2	3	2	1					27	2.16
F_2Asp.×Forcing	pointed	1	1	7	13	21	21	6	4	0	1				75	2.11
„ „	blunt	3	5	6	4	3	1								22	1.43
F_2Berl.×SL	pointed			5	5	11	11	9	6	1	1	3			53	2.40
„ „	blunt			4	2	5	1								12	1.80
F_2K.driv×Altaica	pointed		2	23	38	10									73	1.67
„ „	blunt	2	10	13	1										26	1.32

* Homozygous pointed.
** Heterozygotes.

that the heterozygotes with regard to length/width index resemble the blunt leaved plants rather than the homozygous pointed.

According to HELM (1954) the leaf primordium in lettuce is pointed and the shape of the apex of the adult leaf is determined by the amount of lateral growth of the lamina. It may therefore be assumed that the primary effect of the gene for pointed leaf apex is to restrict lateral growth in the apical part of the lamina sufficiently for the primordial shape of the apex to be retained, and this will automatically have an effect on the length/width index of the lamina. The correlation between pointed apex and length/width ratio may, of course, also be due to linkage between *P* and genes favouring the longitudinal growth of the lamina, or restricting the lateral growth.

5. Venation

The variety Tezier, introduced by the French seed firm Tezier Frères, is charaterized by leaves with a peculiar type of venation. The veins are much more numerous than on normal lettuce leaves and they run parallel almost to the leaf margin with few connecting cross veins. A specimen of Tezier is illustrated in Fig. 11.

This type of venation appears to be the same as that of the 'striate-vein lettuce', described by WHITAKER and BOHN (1953), which occurred as a mutant in a line originating from the cross Imperial A×Best Summer, and which was found to be due to a single recessive gene, *st*. The leaf type of Tezier is also inherited as a simple recessive character and on the strength of the phenotypical similarity it has been assumed that the recessive gene is identical with WHITAKER and BOHN's gene *st*. There was a slight, but significant deficiency in the recessive class (see Table 1) as was also the case in WHITAKER and BOHN's crosses. Among 32 F_3-progenies from normal-veined F_2-plants in the cross Tezier×R.verte, 15 bred true and 17 segregated ($\chi^2_{2:1}=2.449$; P=0.1—0.2) giving a total of 402 normal-veined and 102 striate-veined. Thus there was in these families a deficiency in the recessive class, similar to that found in F_2, and significant at the 5 % level ($\chi^2_{3:1}=6.095^*$; $\chi^2_{het.(16)}=11.979$).

Observations on the relation between striate-vein and other leaf characters were made in the cross Tezier×R.verte. Both parent lines have leaves with a smooth, non-blistered surface, but whereas those of Tezier are curly at the margin those of R.verte are straight. The F_1-hybrids had very curly leaves with a blistered surface. In F_2 there was, among the normal-veined plants, a continuous variation with regard to

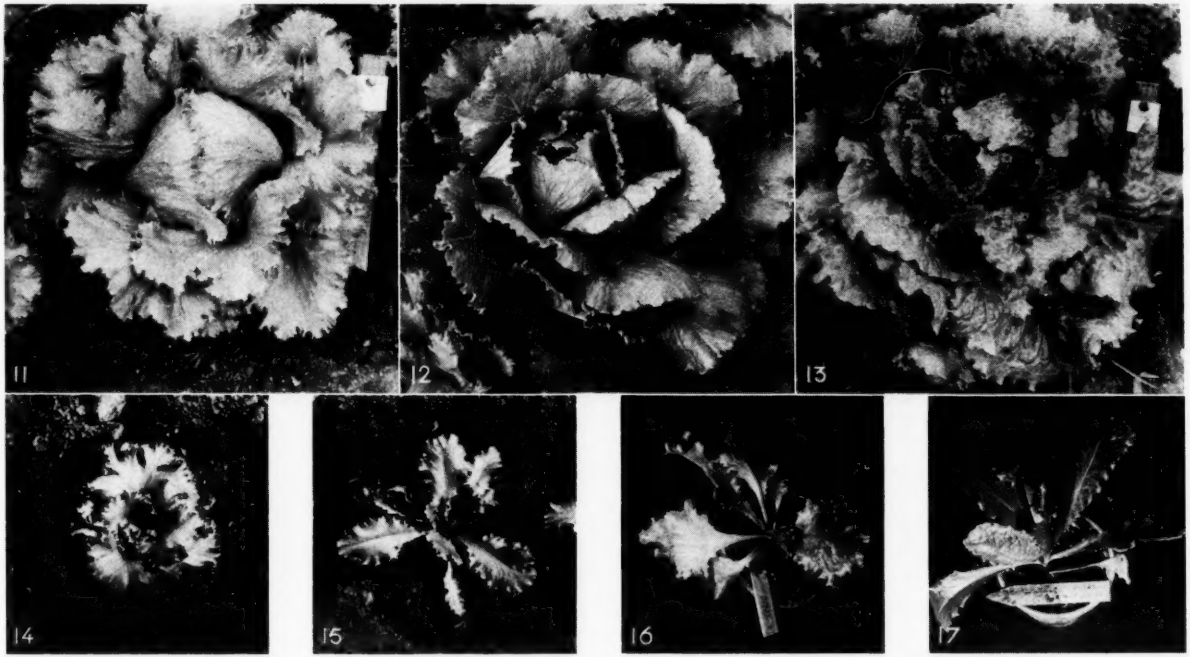
the appearance of leaf surface and margin. The striate-vein plants resembled Tezier in having a smooth leaf surface, but a more or less curly leaf margin. These facts suggest that Tezier has genes for curly margin and blistered surface, but that the effect of these genes is largely suppressed in this variety because of the rigidity imparted to the lamina by the venation pattern.

WHITAKER and BOHN's mutant produced, after selfing, uniform offspring with the same characteristic venation. The variety Tezier, however, does not breed absolutely true for the striate-vein character. A line from this variety has been maintained by single plant propagation for several years. Plants with the normal venation pattern have appeared in most generations as shown by the following data:

Year	Number of plants	
	Total	With normal venation
1952	60	2
1953	20	0
1954	27	0
1955	50	2
1956	116	3
1957	20	0
1958	24	1

The average frequency was 2.5 %. Only plants with the typical striate-vein pattern were in every generation selected for further breeding. Normal-veined plants which have appeared in the variety Tezier are illustrated in Figs. 13 and 16. The normal plants had curly and blistered leaves, and therefore gave the impression of being admixtures. They were, however, uniform and agreed with the striate-vein plants in other characters such as anhocyanin pigmentation, leaf colour, flowering time, seed colour etc. It is therefore most unlikely that their presence could have been due to contamination or accidental cross-pollination. The explanation must be that the recessive gene, *st*, has a tendency to mutate to the dominant allele and that it thus belongs to the category of unstable genes. The genes for curly and blistered leaves, present hypostatically in Tezier, show their effect in the mutants. Seed samples of the variety were obtained from Tezier Frères in 1935 and in 1952 and both contained the same kind of off-type. Progenies have been raised from three normal-veined plants which appeared as mutants, and they all proved to be heterozygous, *Stst*.

In the crosses of WHITAKER and BOHN, striate-veined F_2-plants always gave uniformly striate-veined F_3-families, but Table 10 shows



Figs. 11—17. The variety Tezier and its off-types. — Figs. 11—13. Adult plants. — Fig. 11. Tezier. Fig. 12. The cos off-type. Fig. 13. The normal-veined off-type. Figs. 14—17. Young plants. — Fig. 14. Tezier. Fig. 15. The cos off-type. Fig. 16. The normal-veined off-type. Fig. 17. The normal-veined cos off-type.

that this was not the case in the cross Tezier×R.verte. This table gives the results of F_3, F_4 and F_5-generations produced from striate-veined plants. Among the 17 F_3-families only 3 bred true for the recessive character. Two of these three families were followed through to F_4 and F_5 and produced only striate-vein plants. Three of the families, which gave a proportion of normal-veined plants in F_3, were also followed through to F_5 and in two of them plants with normal venation pattern appeared again in later generations. The F_3-family 30100 was remarkable because the dominant type was found in all the 13 F_5-families which originated from this F_3-family. No such plants appeared in F_4, and the reason was presumably, that the plant numbers in this generation were very small because of a poor seed harvest from the F_3-plants.

The fact that mutations occur in lines originating from some F_3-plants but not from others, must mean that the mutation tendency is genetically controlled. This case resembles that, reported by MÜNTZING (1930, p. 216), in *Galeopsis*, where reversion from the recessive white flower colour to the dominant red occurred in one particular line. Genes which influence the mutation rate of other genes have been identified in some plant species, for instance in maize where the aleurone colour gene a_1 mutates freely in the presence of the dominant gene *Dt*, but not in the presence of the recessive allele *dt* (RHOADES, 1938). In this case the effect of the mutability gene is limited to one specific locus, but in others the general mutation rate is influenced, *e. g.* in the case of the 'Florida mutability factor' in *Drosophila melanogaster* (DEMEREC, 1937). The available data do not permit any definite conclusions about the number of genes controlling the mutation rate of the gene *st* in lettuce, but the fact that a ratio of 14 : 3 was found among the F_3-plants for presence versus absence of mutation tendency, could mean that a single dominant gene is responsible.

F_3-progenies from striate-veined F_3-plants were studied also in the two crosses TTtete×Oakl. and tttete×Fl. 23. They were all found to breed true for the recessive character. Twenty such F_3-families were raised from the cross TTtete×Oakl. and 10 from tttete×Fl. 23. In most cases the number of plants per family was 40 or more. The striate vein line TTtete originates from family 3086 in Table 10 and tttete from family no. 3096. The fact that the recessive type bred true in both crosses is not difficult to understand, if it is assumed that a dominant mutability gene segregates in the cross Tezier×R.verte. On this assumption fam. no. 3086 must be recessive for this gene and is not expected to introduce mutation tendency into its crosses. Fam. no. 3096 contained

one mutant, but may have been segregating for the mutability gene. The plant numbers of the F_4 and F_5-families are too small to show whether this was the case (see Table 10).

The data in Table 10 show that the frequency of mutated gametes must be quite high, about 4.5 % in these families. If the frequency of mutations in somatic cells is of the same magnitude, chimaeras of *St* and *st* tissue ought not to be rare. Such chimaeras have, however, never been found, in spite of a careful search. In this respect the behaviour of the gene *st* differs from that of the flower colour gene in *Galeopsis* (MÜNTZING, 1930) and the aleurone colour gene in maize (RHOADES, 1938) referred to above. These genes mutate in somatic cells and produce chimaeras, as is the case with unstable genes in several other plant species. In order to find out at which stage in ontogeny the mutation *st*→*St* takes place, seeds from 50 seed heads of Tezier were sown out separately. A total of 368 plants were obtained, nine of which were mutants and these nine mutants came from nine different seed heads. This must mean that the mutation takes place at a very late stage in the development of the plant, perhaps even at meiosis.

The sample of Tezier obtained in 1952 produced also a second kind of off-type, namely striate-vein plants with oblong spatula shaped leaves. Such plants are illustrated in Figs. 12 and 15. They did not form proper hearts, but had a growth habit very similar to that of a self-folding cos lettuce variety. This type has since repeatedly appeared in offspring of typical Tezier plants. Attempts to cross the new type with typical Tezier plants have failed so far and therefore it is not known whether its growth habit is due to mutation of a recessive hearting gene as in the case of the rogue in 456 lettuce (PEARSON, 1956). Mutation from *st* to *St* takes place also in the cos off-type and the result is a cos lettuce with normal venation (Fig. 17). It is at present a matter of conjecture whether there is any connection between the occurrence of the two off-types.

In the cross TTtete×Oakl. plants were recovered which had the oakleaved leaf form and were homozygous for *st*. Such plants had a most peculiar appearance. The gene *st* had the effect of drastically reducing the width of the lobes in an irregular manner. Often the lobes were reduced almost to the width of the midrib for part of its length and widened out again on one or both sides towards the apex. Such leaves are illustrated in Fig. 18. The smaller leaves on the upper part of the seed stalk showed hardly any signs of the oak leaf character, but had the appearence of narrow, non-lobed leaves.

TABLE 10. *Breeding results from F_3—F_5 of families originating from stst-plants.*

F_3			F_4			F_5		
Family no.	Number		Family no.	Number		Family no.	Number	
	St	*st*		*St*	*st*		*St*	*st*
3083	2	48						
3084	7	59						
3085	1	12						
			4044	0	1	5001	0	238
3086	0	63				5002	0	53
			4045	0	3	5014	0	28
3087	0	45						
3088	1	20	4046	0	4	5003	0	113
3089	1	42						
3090	12	39						
3092	1	3						
						5004	0	74
						5015	0	106
3093	0	71	4048	0	38	5016	0	51
						5017	0	249
						5018	0	295
3094	7	66						
3095	1	78						
						5005	0	10
3096	1	91	4051	1	7	5019	2	17
						5020	0	6
3097	1	78						
3098	2	77						
3099	4	55						
			4052	0	1			
			4053	0	13	5006	1	27
						5021	5	96
			4055	0	2	5007	3	192
						5024	3	124
						5008	5	148
30100	7	84	4056	0	4	5025	3	46
						5026	14	253
						5009	4	106
						5010	5	225
						5027	4	215
			4057	0	13	5028	1	26
						5029	9	92
						5030	7	128

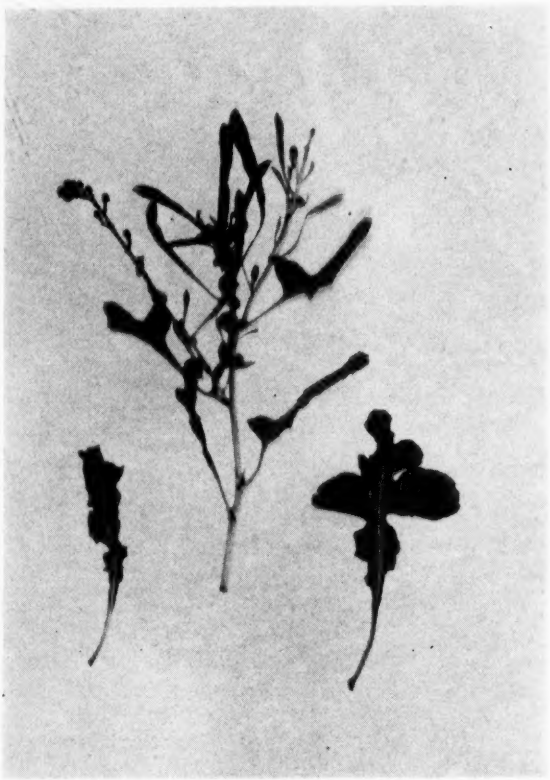


Fig. 18. Leaves and twig of an oakleaved, striate-veined plant.

6. Waxiness

Variation. — The parent lines differed greatly with respect to the degree of wax covering on the epidermis. Thus Altaica is characterized by almost complete absence of wax which gives to the plants a glossy appearance. There is considerable variation between different forms of *L. serriola.* The form from S. Africa (Sc.S.Afr.) has a heavier wax coating than Sc.L, Sc.H and Sc.R. Even more waxy is Sc.int.Sac., a form of *L. serriola integrifolia* which has been received from Sacavem, Portugal. No crosses have, however, been made with this line. Among the varieties of *L. sativa* various degrees of waxiness exist. Those with the most pronounced wax coating have a characteristic dull grey-green

colour, *e.g.* Burpee's Celtuce (*cf.* HELM, 1954). On the other end of the scale there are almost glossy varieties which, however, have slightly but distinctly more wax than Altaica. The parents of the crosses in which the inheritance of this character has been studied can roughly be divided into the following four groups:

1. Heavy wax coating: Sc.S.Afr.
2. Medium „ „ : Sc.L, Sc.H, SL, SP, Asp., Berl., Fl. 23
3. Slight „ „ : K.driv, Laib., Oakl., Laur., Gr. 10
4. Almost glossy : Altaica.

Inheritance. — Presence of wax was dominant in F_1 of all crosses between Altaica and other forms, and clearly monohybrid segregations took place in F_2. The glossy condition in Altaica is apparently caused by a single recessive gene, which will be designated as *gl*. F_2-data showing the segregation of this gene in six crosses between Altaica and more or less waxy forms, have been summarized in Table 1. The agreement with expectation is good.

Variation in F_2 of crosses between the least waxy *L. sativa* varieties and wild forms, and also between *L. sativa* forms of different degrees of waxiness, was almost continuous, and it was not usually possible to classify accurately on a two-class basis. Reasonably clear cut monohybrid segregations were observed in some crosses between *L. sativa* lines with slight and medium wax coating, *e. g.* K.driv×Fl. 23 (slight×medium) and Asp.×Laib. (medium×slight) which gave the ratios 230 : 63 ($\chi^2_{3:1}=1.912$) and 180 : 53 ($\chi^2_{3:1}=0.631$) respectively.

It is not possible to decide on the basis of the data available at present, whether the variation between forms other than Altaica is due to different alleles at the *Gl*-locus or to modifying genes or both.

7. Photoperiodism

Previous results. — Photoperiodism in lettuce and its inheritance has been studied by BREMER (1931) and BREMER and GRANA (1935). These investigations have shown that lettuce varieties differ in their response to day length, some varieties behaving as long-day plants, others as day-neutral plants, and that photoperiodic reaction is inherited in a simple mendelian manner, reaction to long day being dominant. BREMER used the letter *T* for the dominant gene, but in this paper the letter *L* will be employed, as the letter *T* has been used for an anthocyanin gene in accordance with THOMPSON (1938).

BREMER (1931) found that varieties of spring lettuce and winter lettuce are typical long-day plants, whereas varieties of summer lettuce are almost insensitive to differences in photoperiod. The wild species *L. serriola*, *L. virosa* and *L. saligna* were all found to be day-neutral (BREMER and GRANA, 1935).

Classification of parent lines. — Information concerning the photoperiodism of some of the parent lines used in this investigation, has been obtained from three experiments in which the lines were exposed to light periods of different lengths. Experiments 1 and 2 were both conducted out of doors during the summer. Plants receiving long-day treatment were exposed to the natural photoperiod, and the short-day treatment consisted of covering the plants so as to give them only 10 hours of day light. Experiment no. 3 was carried out in the greenhouse during the winter in artificial light, the photoperiods being 18 and 10 hours respectively. Results from the three experiments are presented in Table 11. Because of limited facilities the number of plants per line was small, particularly in experiment no. 1. On the basis of these results and some other observations, the lines have been classified with regard to photoperiodism as follows:

L. sativa lines, cultivated varieties:
Long-day: K.driv, Oakl., Asp.
Day-neutral: Berl., Laur., Asiat., Merv., Paris, R.rouge, Gr. 10

L. sativa lines, primitive forms:
Long-day: Altaica, LC, SP, SL, SB

L. serriola lines:
Long-day: Sc.L, Sc.H, Sc.S.Afr.

The classification was complicated by the fact that the three experiments gave somewhat different results due to different conditions. There was a great difference between the first two experiments with regard to time of sowing. Experiment no. 1 was sown on February 8th and the short-day treatment commenced in the middle of May, whereas experiment no. 2 was sown on June 5th and the short-day treatment introduced as soon as the seedlings emerged. The artificial conditions in experiment no. 3 had an unexpected effect on cultivated as well as primitive forms of *L. sativa*, causing stem elongation to commence at a very early stage irrespective of the light period to which the plants were exposed. This stem elongation did not lead to the initiation of flower primordia and was obviously of a nature different to that, which

TABLE 11. *Length of time between sowing and seed stalk development at long and short days in lines of L. sativa and L. serriola.*

Lines or crosses	Experiment no. 1				Experiment no. 2				Experiment no. 3			
	Long day		Short day		Long day		Short day		Long day		Short day	
	Number of plants	Average number of days	Number of plants	Average number of days	Number of plants	Average number of days	Number of plants	Average number of days	Number of plants	Average number of days	Number of plants	Average number of days
Asp.	5	147	5	170	—	—	—	—	—	—	—	—
Berl.	5	156	4	168	13	84	14	>105	—	—	—	—
K.driv	2	142	3	>172	18	50	8	85	—	—	—	—
Altaica	5	143	3	172	23	50	18	>100	—	—	—	—
LC	—	—	—	—	20	49	16	74	—	—	—	—
SP	5	125	4	133	19	27	17	62	—	—	—	—
SL	—	—	—	—	21	30	20	67	—	—	—	—
SB	—	—	—	—	20	30	18	57	—	—	—	—
Sc.H	5	166	5	>172	20	>105	13	>105	10	76	11	>132
Sc.L	5	168	4	>172	—	—	—	—	36	77	41	>132
Sc.S.Afr.	5	125	5	158	—	—	—	—	9	54	10	121
F_1Berl.×Sc.L	—	—	—	—	—	—	—	—	11	51	12	>132

occurs under natural conditions out of doors. The three *L. serriola* lines, results of which are included in Table 11, did not show this peculiar behaviour.

A few additional comments on the classification of the parent lines are necessary. The line Oakl. has been tried in an experiment in which no exact data were recorded and it was observed to behave as a typical long-day plant. The line Berl. was not absolutely insensitive to long-day treatment, but the response was small compared with a typical long-day line like K.driv. The other *L. sativa* lines, classified as day-neutral, have not been tested in day length experiments, but the classification is based on their lateness in running to seed under normal long-day conditions and their behaviour in crosses. BREMER (1931) found that Kaiser Treib behaved as a long-day variety and that Berliner and Asiatisk were day-neutral.

The three primitive forms SL, SP and SB showed a very clear response to long light period in experiment no. 2, but in experiment no. 1, SP ran to seed very early also in the 10 hr treatment. The difference in behaviour under long day and short day is small for these three lines in comparison with other long-day lines, and they could therefore alternatively be classified as early developing, day-neutral forms. LC could perhaps also be considered as belonging to this category.

Contrary to BREMER's observations, the three *L. serriola* lines behaved as long-day plants, although Sc.L and Sc.H were late in running to seed also under long-day conditions. In experiment no. 2, which was sown very late, Sc.H did not produce any seed stalks in either treatment. The reason for this is not clear. It may mean that plants of this line require to have reached a certain stage of development before they are able to react to long days. The length of day gradually decreased during this experiment and it is possible that the light period in the long-day treatment had fallen below the necessary minimum by the time the plants had reached the stage in their development, at which they would have been able to commence the reproductive phase. It may also mean that exposure to low temperature is necessary for initiation of stem elongation in this line.

F_1-hybrids. — The F_1-hybrids have, as a rule, been grown only under natural conditions of photoperiod. Their behaviour showed that reaction to long day is dominant. F_1-plants of the cross Berl. × Sc.L were included in experiment no. 3 (see Table 11) and showed a response to long-day treatment which was very marked, although less pronounced than that of the parent line Sc.L. In several cases the F_1-hybrid started running

to seed earlier than either parent. Two typical examples of this kind are illustrated by the following data from plants grown side by side in the field:

Lines or crosses	Number of plants with seed stalks at different dates						Total number of plants
	4/6	10/6	17/6	24/6	4/7	10/7	
Berl.	0	0	0	5	12		12
Asp.	0	0	0	11			11
F_1 Berl.×Asp.	13						13
Paris	0	0	0	0	0	9	9
Sc.L	0	0	12				12
F_1 Paris×Sc.L	0	3					3

A further and very striking example is a difference in flowering time observed between the lines Asp. and R.rouge and their F_1-hybrid. All three were included in a randomized block experiment with four replications in which flowering time was recorded. R.rouge required in this experiment 158 days to produce the first flower head, Asp. 159 days, but the F_1-hybrid only 136 days. The difference between R.rouge and the F_1-hybrid was significant at the 0.1 % level. Very similar results were obtained by Mc COLLUM (1953) in crosses between the cultivated variety Prize Head and two different forms of *L. serriola*, the F_1-hybrids coming into flower about two weeks earlier than the parent lines.

These results can be understood if it is assumed that the long-day parents of these crosses have the dominant gene *L* and other genes which to some extent counteract the effect of *L*. In the F_1-hybrid the effect of these genes is suppressed by genes from the day-neutral parent, and the F_1-plants therefore react more readily to long-day conditions. With regard to the data for time of flowering the reservation must be made that, as this is a complex character which depends not only on reaction to photoperiod (*cf.* Mc COLLUM *l. c.*) factors which are irrelevant in this connection may have influenced the results.

F_2-results. — A clear cut segregation occurred in F_2 of crosses between long-day and day-neutral lines of *L. sativa*, and classification presented no difficulties. Data from such crosses are presented in Table 12 and summarized in Table 1. These data amply confirm the monohybrid inheritance of photoperiodism within cultivated forms of *L. sativa*. Crosses between the long-day parents and between the day-neutral parents have proved that the same gene is involved in all the crosses.

When day-neutral *L. sativa* lines were crossed with primitive *L. sativa*

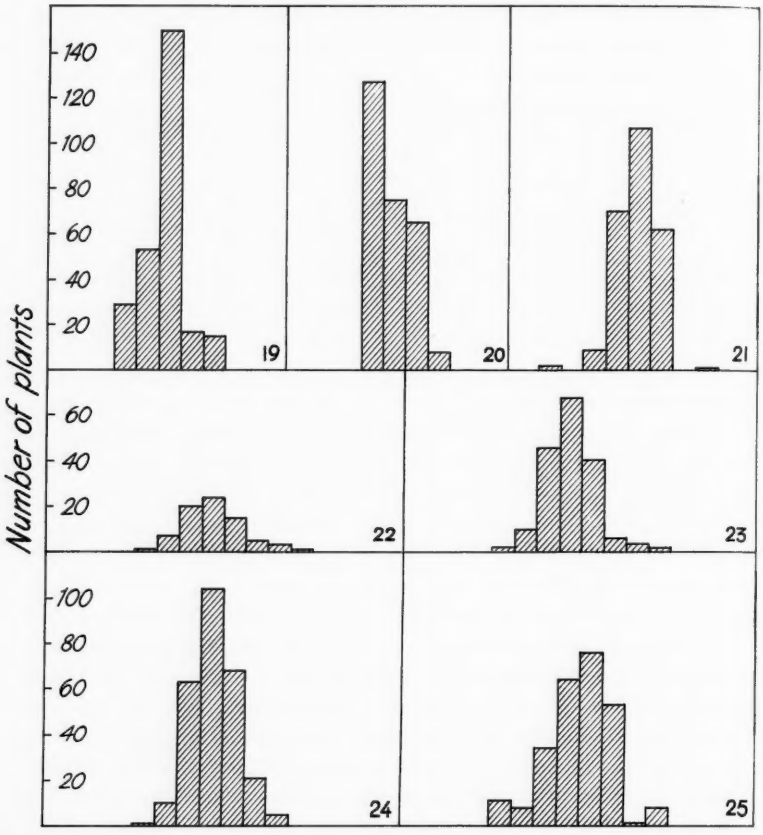
TABLE 12. *F_2-results of crosses with monohybrid inheritance of photoperiodism.*

Crosses	No. of fam.	Number of plants			$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$
		Long-day	Day-neutral	Total		
Berl.×K.driv	4	331	115	446	0.146	2.706
Berl.×Oakl.	7	507	157	664	0.651	12.925
Berl.×Asp.	6	426	153	579	0.627	5.558
Oakl.×Laur.	1	195	66	261	0.011	—
K.driv×Laur.	2	115	46	161	1.096	0.101
Oakl.×Merv.	3	212	76	288	0.296	3.562
K.driv×Merv.	1	37	9	46	0.725	—
Asiat.×Oakl.	3	112	33	145	0.389	0.073
Paris×K.driv	3	218	81	299	0.697	5.859
Paris×Oakl.	1	61	25	86	0.760	—
R.rouge×Oakl.	2	46	16	62	0.022	1.380
K.driv×Gr. 10	3	217	83	300	1.138	6.544
Laur.×Altaica	4	278	79	357	1.570	1.018
Berl.×LC	4	338	125	463	0.986	1.060
Total	44	3093	1064	4157	0.786	

$\chi^2_{\text{het.(13)}}$ for crosses = 8.327

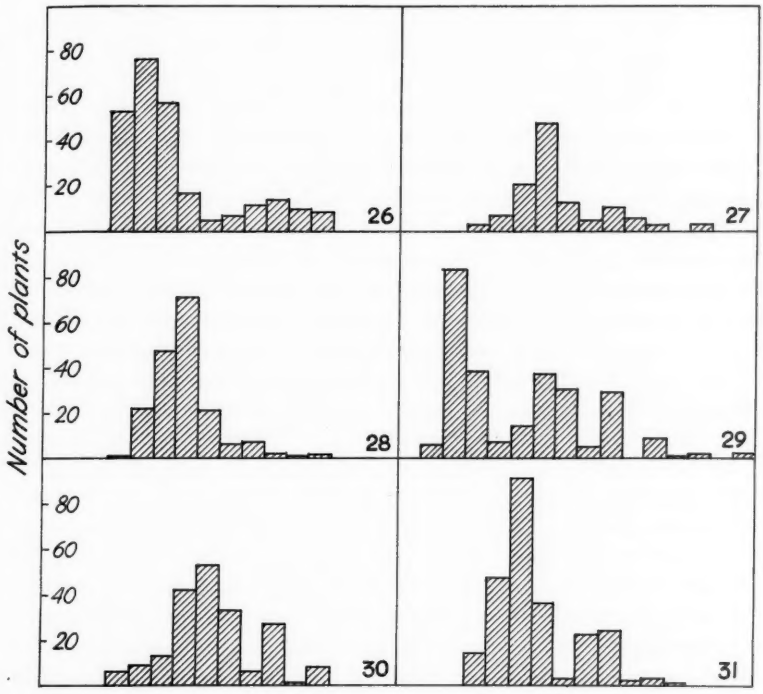
forms or with *L. serriola*, more complicated segregations were, as a rule, found in F_2. Two exceptions are the crosses Laur.×Altaica and Berl.×LC, which were easily classified giving satisfactory monohybrid ratios and the data from these crosses have been included in Tables 1 and 12. F_2-generations of other crosses could not be classified on a two class basis. Instead the date of commencement of stem elongation was recorded for each plant and the data are presented in the form of frequency histograms in Figs. 19—31. Histograms representing the F_2-results of the crosses Berl.×K.driv and K.driv×Asp. have been included for comparison, the former illustrating the wide variation and clearly bimodal distribution caused by the segregation *L—l*, the latter the narrower variation and unimodal distribution in the absence of segregation for these genes.

The variation in F_2 of the crosses K.driv×Sc.H (Fig. 22) and K.driv×Altaica (Fig. 23) is similar to that in K.driv×Asp. (Fig. 19), which indicates that also Sc.H and Altaica have the dominant gene for reaction to long day. The frequency histograms for SL×Altaica, SL×Sc.L, Sc.L×SP and Sc.S.Afr.×Altaica (Figs. 20—21 and 24—25) are also of



Figs. 19—25. Frequency histograms illustrating the variation in F_2 of various crosses with regard to the time required for onset of stem elongation. The width of the columns represents four days. — Fig. 19. K.driv×Asp. Fig. 20. SL×Altaica. Fig. 21. SL×Sc.L. Fig. 22. K.driv×Sc.H. Fig. 23. K.driv×Altaica. Fig. 24. Sc.L×SP. Fig. 25. Sc.S.Afr.×Altaica.

the same type, from which it may by concluded that the gene *L* is present also in SL, Sc.L, SP and Sc.S.Afr. This view is supported by the fact that the variation in F_2 of crosses between Altaica, SP, Sc.H, Sc.S.Afr., and Sc.R and the day-neutral line Berl. is wider than in the crosses mentioned previously as shown by Figs. 27—31. The distribution in these crosses is much less regular than in Berl.×K.driv (Fig. 26), but it shows a definite tendency towards bimodality and may well be the



Figs. 26—31. See explanation to Figs. 19—25. — Fig. 26. Berl.×K.driv. Fig. 27. Berl.×Sc.H. Fig. 28. Berl.×Altaica. Fig. 29. Berl.×SP. Fig. 30. Berl.×Sc.S.Afr. Fig. 31. Berl.×Sc.R.

result of segregation of the gene *L—l*, modified by the segregation of other genes. These data strongly suggest the presence of the gene *L* in lines of *L. serriola* and in the primitive forms of *L. sativa*. Additional evidence that at least one of the *L. serriola* lines has the dominant allele, *L*, was obtained from the cross wwyy×Sc.H. The mother plant of this cross came from an F_2-family of K.driv×Laur., which segregated for the gene pair *L—l*. The F_2-family consisted of 12 plants, 9 *L* and 3 *ll*. One of the nine long-day plants was selected as mother for the cross with Sc.H. The resulting F_1-hybrids were all of the long-day type. Three F_2-families were raised, two consisting entirely of long-day plants, the third containing a proportion of day-neutral plants. The segregation was in this case fairly clear cut and the following data were recorded:

Fam. no.	Long-day	Day-neutral	Total	$\chi^2_{3:1}$
2331	129	29	158	3.722*
2332	185	0	185	
2333	203	0	203	

Although there is a deficiency in the recessive class, the segregation in family 2331 can be accepted as representing a 3 : 1 ratio. It is obvious that either the mother or the father plant of the cross wwyy×Sc.H was heterozygous for a gene controlling the time for onset of stem elongation. As the mother plant is known to come from a family segregating for the alleles *L*—*l*, it is, to say the least, reasonable to assume that these genes were responsible for the observed segregation and from this it follows that Sc.H has the dominant allele at the *L*-locus.

The data presented above indicate that the various lines of *L. serriola* and the primitive forms of *L. sativa*, all have the dominant allele *L*. The great differences which exist between these lines with regard to time of onset of stem elongation under long-day conditions, show that the effect of the gene *L* is influenced by other genes in different ways.

The recessive gene, *l*, for insensitivity to photoperiod, is apparently to be found only in cultivated lettuce. This is contrary to BREMER and GRANA's conclusion (BREMER and GRANA, 1935) that the dominant gene for long-day reaction in lettuce has originated by mutation during domestication. On the contrary, it appears likely that mutation to the recessive allele, *l*, has occurred since lettuce was taken into cultivation. The recessive gene appears to be present only in the type of cabbage lettuce referred to as summer lettuce and in cos lettuce, at least in some varieties. The data in Table 12 show that Paris White Cos and Romaine rouge d'hiver have the recessive gene *l*, and it is reasonable to assume that most cos lettuce varieties have this gene, because they run to seed about the same time as the day-neutral summer varieties of cabbage lettuce, when grown out-doors during the summer.

8. Hearting

Variation. — With the exception of the asparagus or stem lettuce which is grown for the sake of its stems, all varieties of garden lettuce are cultivated as sallad plants and have therefore been subjected to continuous selection for edible, tender leaves. The most tender and palatable leaves are produced when, at the rosette stage, the young leaves in the middle become enfolded by the outer, older leaves. Developing in protection and darkness, these young leaves become

bleached, brittle and succulent as is the case in cabbage lettuce and in cos lettuce varieties of the self-folding type. In cabbage lettuce varieties the young leaves form, at an early stage, an open rosette. The folding process, which is due to an inward curving of midribs and veins (*cf.* CHODAT and GAGNEBIN, 1948), begins with the young central leaves and proceeds outwards to the older leaves. Hearting in cabbage lettuce varieties in thus a gradual process and the hearting tendency can be observed at an early stage. The stronger the curving tendency of midrib and veins, the firmer is the resulting heart. In self-folding cos lettuce varieties the young plants remain as open rosettes for a longer period, and the folding is achieved mainly by the curving of midribs and veins of the outer, older leaves. The folding tendency is entirely lacking in some cos varieties, in which it is necessary to secure the outer leaves in an upright position by tying, in order to achieve the desired bleaching effect, but such varieties have now practically gone out of cultivation. Leaf lettuce varieties do not form hearts, only more or less compact bunches of leaves. No hearts are formed by asparagus lettuce, any of the primitive forms of *L. sativa* or any wild lettuce species, including *L. serriola.*

Short internodes are obviously essential for heart formation, and any factor encouraging stem elongation is therefore detrimental to hearting. Photoperiodicity, temperature, moisture and soil conditions are important in this respect. Long-day varieties of cabbage lettuce, whilst producing good hearts when grown under glass during winter and spring, run to seed without forming hearts when grown in the open during the summer. The influence of temperature on stem elongation is also very important, and genetical differences exist in reaction to high temperatures (THOMPSON, 1951). Drought and unfavourable soil conditions can also have the effect of inducing early stem elongation and so interrupt the vegetative development of plants, potentially capable of forming hearts.

Previous results. — From the above it is clear that the process of heart formation depends on a rather complicated interaction between genotype and environment. Inheritance of hearting in lettuce has been studied by DURST (1930), LEWIS (1930), BREMER and GRANA (1935) and PEARSON (1956). DURST (*l. c.*) concluded on the basis of results from crosses involving forms of *L. serriola*, a leaf lettuce variety, a cos lettuce variety and two varieties of cabbage lettuce, that hearting is inherited in a quantitative manner. LEWIS (1930), who worked mainly with crosses between different varieties of cabbage lettuce, arrived at the

same conclusion. BREMER and GRANA (1935) found monohybrid segregations with non-hearting as the dominant character, in crosses between cabbage lettuce varieties and 'Kurztagrosette I', a non-hearting mutant which appeared in the variety Kaiser Treib (syn. Emperor). They introduced the symbols *K* and *k* for the allelic pair. No plants with the hearting habit were recovered in F_2 of their crosses between various forms of *L. serriola* and cabbage lettuce, but the significance of this cannot be assessed as no information is given about the size of the F_2-families. PEARSON (1956) analyzed a non-hearting rogue type which regularly appears, with an average frequency of about 1 %, in a cabbage lettuce variety known as 456, and he concluded that the rogue originates through mutation of a recessive gene for hearting to its dominant allele. This is a parallel to the origin of BREMER's 'Kurztagrosette'. PEARSON used the letter K for the dominant gene in 456, *i. e.* the letter introduced by BREMER for the dominant in his mutant from Kaiser Treib, but it has not been proved that the genes are identical.

The fact that the difference between non-hearting plants in BREMER's and PEARSON's material was controlled by a single pair of genes does, of course, not contradict the conclusions reached by DURST and LEWIS that the hearting habit in their material was inherited in a quantitative manner. The habit of growth in the rosette stage is apparently controlled by a number of genes which are different in different forms. Additional information about the inheritance of hearting has been obtained in the course of the present investigation.

Classification. — Self-folding cos varieties have been classified as non-hearting and all hearting parent lines therefore are derived from cabbage lettuce varieties. Non-hearting parent lines were Paris, R.rouge. R.verte and Gr. 10, all originating from self-folding cos varieties, Oakl., a leaf lettuce type forming dense rosettes, Asp., an asparagus lettuce with funnel-shaped rosettes and a tendency towards self-folding, several lines originating from primitive *L. sativa* forms and from *L. serriola* and all forming open, more or less prostrate rosettes and finally the line K.driv which requires special comment.

This line originates from a non-hearting plant found in the long-day variety Kejsar driv which is synonymous with Kaiser Treib, the variety in which BREMER and GRANA (1935) found the 'Kurztagrosette I'. It is reasonable to assume that both lines owe their non-hearting characteristics to mutation $k \rightarrow K$, although the relationship between the two stocks of the variety is unknown. The spontaneous occurrence of non-hearting plants in cabbage lettuce varieties is not a rare event. The case of the

rogue in lettuce 456 has already been referred to. LENANDER (1939) found varying proportions of non-hearting plants in different strains of the variety Maikönig and a similar case has been observed by the writer in the variety Merveille des quatre saisons.

Hearting is not the most favourable of characters for genetical analysis. Whilst the majority of the plants in segregating families can readily be classified as hearting or non-hearting, plants are often encountered which are difficult to place in one class or the other. Some of these are plants, which in the early stages showed tendency towards hearting, but in which the vegetative development was interrupted by early onset of stem elongation. Others were plants which, although they appeared to have had time for full vegetative development, produced a bunch of leaves in which only the youngest showed the characteristic folding habit and curvature of the midrib. In certain crosses the plants have been classified into three groups, non-hearting, hearting and intermediate. When comparing with expected ratios, the intermediate group was counted as hearting in order to obtain a maximum figure for the frequency of hearting plants, and as non-hearting in order to obtain a minimum figure. In view of the influence of photoperiodism on the vegetative development it would, of course, have been desirable to grow the material under short-day conditions in order to allow all the plants time for full vegetative development. This was impossible for practical reasons, but it was found that plants raised in the greenhouse during February and March and transplanted to the field during April, in most years had suitable conditions and sufficient time for full vegetative development before the commencement of stem elongation (*cf.* CHODAT and GAGNEBIN, 1948). There were, however, exceptions to this, particularly in crosses involving the forms SL, SP and SB, which are characterized by very early onset of stem elongation, a fact which, no doubt, has influenced the results.

Evidence for the existence of the genes K and H. — F_2-data are presented in Table 1 for two genes, *K* and *H*, affecting habit of growth in the rosette stage. The results for the gene *K* come from a single cross, Berl.×K.driv, and the identity of the dominant gene from K.driv and BREMER and GRANA's gene *K*, has been assumed on the strength of the similarity in mode of origin between K.driv and BREMER and GRANA's 'Kurztagrosette I' (BREMER and GRANA, 1935). The gene *H* segregated in crosses between cabbage lettuce lines and Altaica, Paris, R.verte and Gr. 10. An F_3-generation consisting of progenies from 13 non-hearting F_2-plants in the cross Gr. 10×Laur. gave the following results:

Number of families		Total of segregating families			
Constant non-hearting	Segregating	Non-hearting	Hearting	$\chi^2_{3:1}$	$\chi^2_{\text{het. fam.}}$
4	9	282	71	4.496*	12.254

Each family consisted of 26 or more plants. The results confirm the monohybrid segregation found in F_2, although there is a slight deficiency of hearting plants in segregating families. The non-identity of the genes *H* and *K* is proved by the following data which show that hearting plants occur in F_2 of crosses between K.driv and Altaica, Paris and Gr. 10 in proportions which agree fairly well with a 15 : 1 ratio.

	Non-hearting	Hearting	Total	$\chi^2_{15:1}$	Number of families	$\chi^2_{\text{het. fam.}}$
K.driv×Altaica	670	38	708	0.942	9	3.355
Paris×K.driv	273	12	285	2.023	3	1.030
K.driv×Gr. 10	72	1	73	2.967	1	—
	1015	51	1066	3.909*		
			$\chi^2_{\text{het.(2)}}$ for crosses:	2.023		

Further proof that the two genes are not identical is provided by different linkage relations with the gene *g*, for yellow leaf colour which is present in Berliner. The data below show that the gene present in Altaica and Gr. 10 is linked to the *G*-locus, whereas the gene in K.driv is inherited independently of this locus. The linkage χ^2 for Berl.×K.driv is also significant at the 5 % level, but this is due to a deficiency in the class of the double recessive, which cannot be caused by linkage as the cross is of the coupling type.

	Non-hearting		Hearting		Total	χ^2_L
	G	*g*	*G*	*g*		
Berl.×K.driv	371	116	131	19	637	6.908*
Berl.×Altaica	311	45	24	75	455	148.191***
Berl.×Gr. 10	244	35	35	44	358	57.387***

In agreement with expectation no hearts appeared among 283 F_2-plants of the cross Paris×Altaica, both parent lines being *kk HH*.

Two crosses between cabbage lettuce and self-folding cos lettuce, namely R.verte×Merv. and Paris×B.Bost., produced open rosettes in F_1, which proves that the genes for the self-folding habit in cos varieties are not the same as those for the hearting habit in cabbage lettuce. Plants showing varying degrees of self-folding tendency were observed in the non-hearting class in F_2, but it was not possible to divide the plants into

groups with regard to this character and no information was gained about the inheritance of the self-folding habit.

Crosses involving two genes for hearting. — The data presented below show that the line Oakl., when crossed with cabbage lettuce, in F_2 gives 15 : 1 ratios, although with a slight deficiency in the recessive class. Oakl. must therefore have two dominant genes for rosette habit.

	Non-hearting	Hearting	Total	$\chi^2_{15:1}$	Number of families	$\chi^2_{\text{het. fam.}}$
Berl. × Oakl.	545	25	570	3.380	6	6.668
Asiat. × Oakl.	136	9	145	0.000	3	0.746
Oakl. × Laur.	245	16	261	0.006	1	—
Oakl. × Merv.	276	12	288	2.133	3	0.002
Total	1202	62	1264	3.902*		

$\chi^2_{\text{het.(3)}}$ for crosses: 1.592

In the cross Berl. × Oakl. evidence was found of linkage between the leaf colour gene *G*, and one of the segregating genes for hearting, the dihybrid segregation ratio being 422 : 121 : 14 : 11, and the χ^2 for linkage 6.319*. One of the segregating genes is therefore *H*, but whether the other is *K* must be an open question.

The crosses considered so far have been classified on a two class basis without difficulty. There remain a number of crosses in which the segregation was less clear cut, and in which it was necessary to introduce a class intermediate between non-hearting and hearting. However, a certain amount of information is to be gained also from these crosses.

F_2-results of crosses between cabbage lettuce and the lines Asp., LC, SP and SB are presented in Table 13. The data show that the segregation ratios are intermediate between 15 : 1 and 63 : 1, if only well developed hearts are included in the recessive class, but they agree with 15 : 1 if intermediates are also counted as hearts.

The non-hearting parents of these crosses are all of the long-day type and particularly SP and SB run to seed very early. It is possible that in some F_2-plants, potentially capable of forming hearts, stem elongation started before the vegetative development was completed, and such plants may have been classified as rosettes. There is in fact evidence that this has been the case. The segregation in time of onset of stem elongation was such that the plants could readily be divided into an early and a late group. The following data are based on the strict classification, intermediates having been counted as non-hearting. They

TABLE 13. *Inheritance of hearting; F_2-data from crosses involving two or possibly three genes.*

Crosses	Number of families	Number of plants	Intermediates classified as hearting					Intermediates classified as non-hearting				
			Non-hearting	Hearting	$\chi^2_{15:1}$	$\chi^2_{63:1}$	$\chi^2_{het.\ fam.}$	Non-hearting	Hearting	$\chi^2_{15:1}$	$\chi^2_{63:1}$	$\chi^2_{het.\ fam.}$
Berl.×Asp.	6	582	550	32	0.561	58.614***	5.352	567	15	13.397***	3.897*	2.784
Berl.×LC	4	467	443	24	0.983	38.842***	6.665	455	12	10.796**	3.079	16.534
Berl.×SP	2	260	242	18	0.201	48.575***	0.016	253	7	5.616*	2.158	0.681
SB×Maik.	2	282	257	25	3.291	97.778***	0.074	274	8	5.607*	2.978	0.279
		1591	1492	99	0.003			1549	42	35.389***	12.006***	
			$\chi^2_{het.(3)}$ for crosses = 5.033					$\chi^2_{het.(3)}$ for crosses[1] = 0.052				

[1] POISSON formula. See page 388.

show that the ratios are in good agreement with 15 : 1 in the late class, whereas there is a significant deficiency of hearting plants in the early class.

	Early			Late		
	Non-hearting	Hearting	$\chi^2_{15:1}$	Non-hearting	Hearting	$\chi^2_{15:1}$
Berl.×Asp.	216	4	7.375**	72	4	
Berl.×LC	332	5	13.066***	117	7	
Berl.×SP	199	3	7.827**	54	4	
SB×Maik.	235	4	8.543**	39	4	
Total	982	16	36.778***	282	19	0.002
	$\chi^2_{het.(3)}$ for crosses [1]		0.166			0.859

[1] The POISSON formula.

There is thus reason to believe that only two genes for hearting are involved in these crosses, although similar results are, of course, to be expected, if three genes are segregating, one of which is linked to a gene for late onset of the reproductive phase.

There is a clear indication of linkage between hearting and yellow leaf colour in the crosses Berl.×Asp., Berl.×LC and Berl.×SP, which together give a segregation ratio of 799 : 197 : 11 : 16 on the stricter classification for hearting (χ^2_L=24.854***). The dominant gene *H* is apparently present in Asp., LC and SP.

. *Crosses involving more than two genes.* — The data in Table 14 show that very few hearts appeared in F_2 of six crosses between cabbage lettuce and the four lines SL, Sc.H, Sc.R and Sc.S.Afr. Heterogeneity between crosses, tested with the χ^2 method suggested by Dr. BLOM (see page 388) is highly significant when the liberal classification for hearting is applied. This is due to the two crosses Berl.×Sc.R and Berl.×Sc.L which have high proportions of plants classified as hearts compared with the other crosses. When the stricter classification is applied the total of the six crosses agrees with a 255 : 1 ratio, and the χ^2 for heterogeneity is not significant.

Also in these six crosses there is evidence of linkage between hearting and yellow leaf colour. The total of the five crosses in which the yellow leaved line Berl. took part, gives with the more discriminating classification for hearting a segregation ratio of 1965 : 322 : 3 : 9 (χ^2_L= 35.947***).

It was shown in connection with inheritance of leaf colour that segregation of the leaf colour genes *G* or *Lg*, is seriously disturbed in the crosses Berl.×SL, Berl.×Sc.H, Berl.×Sc.S.Afr. and Maik.×Sc.S.Afr.,

TABLE 14. *Inheritance of hearting; F_2-data from crosses involving more than two genes.*

Crosses		Number of families	Number of plants	Classification				
				Liberal			Strict	
No.	Parents			Non-hearting	Hearting	$\chi^2_{15:1}$	Non-hearting	Hearting
1	Berl.×SL	6	499	493	6		498	1
2	Berl.×Sc.H	12	865	858	7		863	2
3	Berl.×Sc.R	2	242	235	7	4.656*	240	2
4	Berl.×Sc.S.Afr.	6	382	375	7		380	2
5	Berl.×Sc.L	3	311	295	16	0.648	306	5
6[1]	Maik.×Sc.S.Afr.	9	666	655	11		663	3
		Total	2965				2950	15

$\chi^2_{het.(5)}$ for crosses[2]=20.934*** 7.586 (P=0.10—0.20)

$\chi^2_{63:1}$ =21.521***

$\chi^2_{255:1}$= 1.013

[1] Including the reciprocal cross.

[2] POISSON formula. See page 388.

but normal in Berl.×Sc.L and Berl.×Sc.R and that the disturbed segregations are associated with partial pollen sterility. As *G*, *Lg* and *H* are situated on the same chromosome this abnormality must influence also the segregation *H*—*h*, but the resulting deficiency of hearting plants cannot be estimated accurately. Therefore only F_2-results from the crosses Berl.×Sc.R and Berl.×Sc.L are suitable for estimation of the number of segregating genes for hearting. With the stricter classification these two crosses give a total of 546 non-hearting and 7 hearting plants, which is a good fit for at 63 : 1 ratio ($\chi^2_{63:1}=0.316$; $\chi^2_{het.}=0.722$). With the more liberal classification Berl.×Sc.L has given a segregation which can be accepted as agreeing with a 15 : 1 ratio, although with a deficiency of hearting plants, but the ratio found for Berl.×Sc.R differs significantly from 15 : 1. The conclusion to be drawn from these data is that Sc.R has three dominant genes for the non-hearting habit and that this is probably true also for Sc.L.

The results of crosses no. 1, 2, 4 and 6 in Table 14 have been compared with expectation on the assumption that only two genes, one of which is *H*, are involved in the segregation, and that the segregation ratio for *H*—*h* in each cross is the same as the ratio observed for the leaf colour genes. The following results were obtained:

	Expected	Observed	χ^2
Berl.×Sc.H	844.00 : 21.00	858 : 7	8.895**
Berl.×Sc.S.Afr.	371.50 : 10.50	375 : 7	0.881
Berl.×SL	478.25 : 20.75	493 : 6	10.211**
Maik.×Sc.S.Afr.	646.43 : 19.57	655 : 11	3.863*

The observed numbers refer to the liberal classification, intermediate plants having been counted as hearts, but in spite of this the observed numbers of hearts are lower than the expected numbers in all four crosses and the difference is significant in three of them. This suggests that also the lines Sc.H, Sc.S.Afr. and SL have three dominant genes for the non-hearting condition.

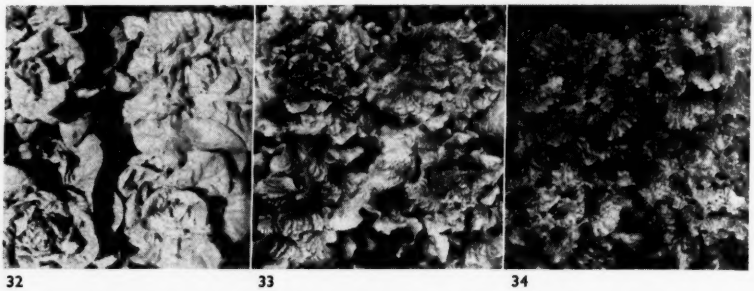
F_3-data are available of the cross Berl.×Sc.R for 49 families from non-hearting F_2-plants. The number of plants, which varied from 34 to 60, is in many families too small to give the recessive type a reasonable chance of appearing in case of 15 : 1 or 63 : 1 segregations. The families are, however, sufficiently large to allow the recognition of families segregating 3 : 1. In 32 of the families all plants were non-hearting, in 13 a few more or less developed hearts appeared, but in four families about one quarter of the plants formed hearts. These four families gave the following segregation ratios:

Non-hearting	Hearting	$\chi^2_{3:1}$
44	10	1.210
50	10	2.222
27	7	0.354
52	7	5.429*

The segregation in the last family is a poor fit for 3 : 1, but in all these families the strict classification has been applied and in spite of the result of the χ^2-test, it is likely that the ratio in this family represents a 3 : 1 segregation.

Among 49 families, the numbers which are expected to show monohybrid segregation are, with two genes involved 13.1, with three genes involved 4.7 and with four genes 1.5. The observed number of 3 : 1 families therefore agrees best with trihybrid inheritance and this confirms the results from the F_2-generation. There can thus be little doubt that a third dominant gene for the non-hearting condition exists in some lines of *L. serriola.* This gene will be designated as *Ca.*

Modifying genes. — The existence of genes, modifying the effect of the major genes for hearting, is well illustrated by the results of the cross Berl.×Montrée. The variety Montrée jaune frisée à bords rouges,



32 33 34

Figs. 32—34. Growth habit in Berl., Montrée and F_1Berl.×Montrée. — Fig. 32. Berl.; well developed hearts. Fig. 33. F_1; rosettes. Fig. 34. Montrée; loose hearts.

from which the line Montrée originates, is capable of forming loose hearts under favourable conditions, but often it produces only a thick bunch of leaves. It is, in fact, intermediate between leaf lettuce and cabbage lettuce. When crossed with the cabbage lettuce line Berl., the line Montrée gave F_1-hybrids, which produced wide open rosettes without any tendency towards hearting. Parent lines and the F_1-hybrids are illustrated in Figs. 32—34.

The F_2-generation consisted of a great variety of types ranging from rosettes, even more wide open than those of the F_1-hybrids, to hearts, equally good as those of the parent variety Berl. A visual scale was adopted for classification, ranging from -3 to $+3$. The designations of the classes have the following meaning:

Class	Growth habit
−3	Prostrate rosette
−2	Semi-upright rosette
−1	Upright rosette
0	Bunch of leaves
+1	Loose, poorly developed heart
+2	Fairly well developed heart
+3	Firm, well developed heart

Results of the classification of parent lines, F_1 and F_2 are presented in Table 15. In this table are also found data from backcrosses between F_1 and the two parents. They show that the backcross with Berl. gave a clearly bimodal distribution with one peak at -2, the other at $+2$, whereas the back cross with Montrée gave a continuous distribution ranging from -3 to $+1$.

In order to explain these results it has been assumed that Montrée has

TABLE 15. *Inheritance of hearting in the cross Berliner × Montrée.*

Parent lines or crosses	Fam.	−3	−2	−1	0	+1	+2	+3	Total	Non-hearting	Hearting	$\chi^2_{3:1}$	$\chi^2_{15:1}$
Berliner 1st year							1	16	17		17		
„ 2nd year								10	10		10		
Montrée 1st year					12	5			17	12	5		
„ 2nd year					6	8			14	6	8		
F_1 Berliner × Montrée 1st year			15							15			
„ „ 2nd year			2							2			
F_2 Berliner × Montrée	2002	14	52	15	10	5	4	4	104	91	13		
„ „	2003	26	66	20	3	1	3	1	120	115	5		
„ „	2101	2	21	8	4	1	0	0	36	35	1		
„ „	2102	1	39	5	1	2	2	0	50	46	4		
	Total	43	178	48	18	9	9	5	310	287	23		0.723
„ „	2103	1	31	42	19	15	10	8	126	93	33	0.095	
Backcross Berliner × F_1		5	27	23	16	5	14	11	101	71	30	1.191	
„ Montrée × F_1		2	46	85	50	6	0	0	189	183	6		

the dominant alleles at the *K* and *H* loci and that it owes its weak hearting capacity to an unknown number of recessive modifying genes. Berl., which has the recessive alleles *k* and *h*, has dominant alleles for the modifyers. One of the major genes shows linkage with the leaf colour gene *G* (see below), and therefore must be identical with *H*. There is no proof that the other gene is identical with *K*, but this will be assumed for the present.

When considering the agreement between the hypothesis and the observed segregations, plants belonging to the classes +1, +2 and +3 have been counted as hearting and the rest, including class 0, as non-hearting. According to the hypothesis the F_1-hybrids have the genotype *KkHh*. The rosette habit is accounted for by the fact that they have received from Berl., dominant genes which suppress the effect of the modifiers. The backcross Berl.$\times F_1$ represents the cross *kkhh*$\times$*KkHh* and is expected to give an approximate 3 : 1 ratio. The numbers obtained are 71 non-hearting: 30 hearting, which is a good fit ($\chi^2_{3:1}=1.191$).

The difficulty of ensuring 100 % hybrids in lettuce backcrosses has been referred to earlier in this paper. A large number of selfs in the cross Berl.$\times F_1$ could account for the difference between this cross and the backcross Montrée$\times F_1$. However, a careful examination of other characters revealed that only 8 of the 101 plants could have been the result of selfing. If these 8 plants are discarded, the ratio becomes 71 : 22, which is actually an even better fit for 3 : 1 ($\chi^2=0.896$). Only the modifiers are expected to affect the segregation in the backcross between F_1 and Montrée. The observed continuous variation is in agreement with this expectation. The data give no information about the number of genes involved. Some of the plants in the two classes 0 and +1 may have originated through selfing, although only one plant was recorded as agreeing in all respects with the mother plant, Montrée.

A 15 : 1 ratio with some excess of hearting plants because of the segregating modifiers is, according to the hypothesis, to be expected in F_2 of the cross Berl.$\times$Montrée. The four first families agree with this expectation giving a total of 287 : 23 ($\chi^2_{15:1}=0.723$), but the fifth F_2-family, no. 2103, has a much higher proportion of hearts than the others, and it also has the greatest number of non-hearting plants in class −1 instead of −2. The ratio between non-hearting and hearting plants is 93 : 33, which agrees with 3 : 1, not with 15 : 1 ($\chi^2_{3:1}=0.095$).

The two families 2102 and 2103 had both parent plants in common. The discrepancy between the families can be explained only by assuming, that the Montrée-plant used as father for the cross, was

heterozygous at the *K* or *H*-locus. There was clear evidence of linkage between hearting and leaf colour in family no. 2103 as shown by the following data:

Non-hearting		Hearting		Total	χ^2_L
G	*g*	*G*	*g*		
77	16	15	18	126	18.797***

As it is known that the *G*-locus is linked with the *H*-locus, this result shows that it is the gene pair *H*—*h* which segregates in this family, and from this the conclusion can be drawn, that the Montrée plant used as father must have been heterozygous at the other locus, which presumably is the *K*-locus. This is unexpected as the line Montrée is very uniform morphologically, which makes it unlikely that the heterozygosity is due to recent hybridization. A possible explanation is, that mutation of a gene for hearting, probably *k*, has taken place in the variety Montrée, as has been shown to be the case in Kaiser Treib and 456 Lettuce (see page 432). The visible effect of such a mutation must in this case be expected to be small because of the presence of the dominant *H* gene.

Progenies varying in size between 31 and 43 individuals, and originating from 34 F_2-plants in families 2101 and 2102, were raised and classified in the same way as the F_3-generation. It seems unnecessary to present the results in full, but the distribution of the families with regard to percentage of hearting plants is given below.

	Percentage of hearting plants															
	0	1	4	8	12	16	20	24	28	32	36	40—52		56—84	88—96	100
Number of families	9	3	4	5	2	1	1	2	3	—	1	1		1	2	

From this it can be seen that the frequency distribution for segregating families has two maxima, one between 8 % and 12 %, the other between 28 % and 32 %. This strongly suggests the existence of the two kinds of segregating families, which are to be expected if the segregation is dihybrid. The effect of the modifying genes may account for the fact that the number of non-segregating families is lower than expected (9 against 14.9). Another reason for this may be the small number of plants per progeny. The three families with the highest percentages of hearts originated from F_2-plants belonging to class +1. One family, originating from an F_2-plant in class −1, had as many as 55 % of hearting plants. This family may be assumed to segregate for one of the major gene pairs and have the recessive allele of the other, and further to have a number of modifiers making the heterozygotes into hearts.

A number of F_3-families were raised also from F_2-family 2103 and they gave the following distribution with respect to plants classified as hearts:

	Percentage of hearting plants														
	0	1	4	8	12	16	20	24	28	32	36	40—56	60—96	100
Number of families	1	—	—	2	1	—	1	3	1	1	—	—	1	—	2

This distribution is entirely different to that found in progenies from families 2101 and 2102 and it confirms the monohybrid segregation in family 2103. Five of the families originated from hearting F_2-plants (class +1). Two of these gave between 96 and 100 % hearts, but the other three gave only 20 %, 25.5 % and 31.0 % of hearts respectively. In these three cases the F_2-plants may have been heterozygotes modified into hearts.

Crosses between non-hearting lines. — Hearting plants appeared in F_2 of crosses between non-hearting lines only when K.driv was one of the parents and Altaica, Paris or Gr. 10 the other. These crosses have been discussed above on page 434. F_1 and F_2 of a further 25 crosses between non-hearting lines have been grown, but they produced only non-hearting plants which shows that the parent lines had at least one dominant gene in common. A reservation must, however, be made for one of the crosses, K.driv×SL, which gave one doubtful heart among 266 F_2-plants.

Crosses between hearting lines. — Most crosses between lines from cabbage lettuce varieties with proper hearting qualities have given F_1 and F_2 generations consisting entirely of more or less well developed hearts. An exception is the cross Berl.×Wond. The F_1-generation of this cross consisted of rather poor hearts (class +1), although both parents form hearts of excellent quality. In F_2 there was a large proportion of non-hearting plants as shown by the following data:

Leaf colour	Habit of growth						Total	
	−2	−1	0	+1	+2	+3	Non-hearting	Hearting
Green	3	43	9	12	24	8	55	44
Yellow		7	2	1	11	6	9	18
Total	3	50	11	13	35	14	64	62

The observed numbers of non-hearting and hearting plants agree fairly well with a 9 : 7 ratio. Segregation of the leaf colour genes *G* and *g* also took place in this cross, and the data indicate an association between hearting tendency and yellow leaf colour. This suggests that the gene *H* is involved in the segregation for hearting, as this gene is known

to be linked to *G*. That would mean that the variety Wonderful has the dominant gene at the *H*-locus and owes its hearting habit to recessive genes not present in Berliner, but more data are desirable before definite conclusions can be drawn.

LEWIS (1930) found varying proportions of non-hearting plants in F_2 of most of his crosses between hearting varieties. It is in agreement with the above results that these proportions were particularly high in those crosses in which the variety New York, which is synonymous with Wonderful, was one of the parents.

Conclusions. — The data presented above prove the existence of three major genes for hearting in lettuce. This does, however, not invalidate the conclusion reached by DURST (1930) and by LEWIS (1930), that hearting tendency is inherited in a quantitative manner, because it is obvious that there are, in addition to the major genes, a number of modifying genes. This was brought out particularly clearly in the cross Berl. × Montrée, which in F_2 gave an almost continuous range of types from open rosettes to hearts. The self-folding habit of cos lettuce varieties is due to recessive genes — or possibly one recessive gene — not identical with *k* or *h*.

A summary is given below of the most likely number of dominant genes in the different non-hearting parent lines:

Number of dominant genes			
3	2	1	
		K	*H*
SL	Oakl.	K.driv	Altaica
Sc.R	Asp.		Paris
Sc.H	LC		R.verte
Sc.L	SP		Gr. 10
Sc.S.Afr.	SB		

9. Spination

Variation. — *L. serriola* owes its common name 'Prickly Lettuce' to the presence of prickles or spines on leaves and stem. The degree of spination shows great variation. Some forms, in this material represented by Sc.S.Afr., have numerous spines on the whole length of the seed stalk, and on the dorsal side of the leaves spines are present on the smaller veins as well as on the midrib, which usually has two rows of spines set at an angle to each other. In other forms of *L. serriola*, represented by Sc.H, Sc.L and Sc.R, the smaller veins have no spines and the midrib has only one row, which continues a short way down

on the stem. Apart from this the seed stalk has few spines except at the base, where they are usually numerous. Completely non-prickly forms of *L. serriola* are also known, such as the non-lobed form to which ALLIONI gave the name *L. augustana* (*cf.* LINDQVIST, 1960 c).

In cultivated forms of *L. sativa*, spines are completely or almost completely lacking. The primitive *L. sativa* forms, however, show the same variation as *L. serriola*. Forms with very strong spination are represented by the line SB, forms with moderate spination by SL and SP and non-prickly forms by LC and SM. The latter line, which is very similar to SB, SL and SP, has, however, not been used in any crosses. Altaica differs from other lines in this material in having spines on midrib only and none at all on the seed stalk. It agrees in this respect with *L. saligna* rather than with any form of *L. serriola* or *L. sativa*.

Inheritance. — Inheritance of spination has been studied by DURST (1930) in crosses between *L. serriola* and cultivated varieties of lettuce. He found that a single pair of genes, *S*—*s*, determines presence or absence of spines, presence being the dominant character. The monogenic inheritance of this difference has been amply confirmed in a large number of crosses in the course of this investigation and the results are summarized in Table 1. The dominant gene present in *L. serriola*, is responsible also for the spination of the two primitive *L. sativa* forms SP and SL, a fact which is proved by the following F_2-data:

Crosses	Segregation in F_2			$\chi^2_{3:1}$
	Prickly	Non-prickly	Total	
Berl.×Sc.L	232	73	305	0.185
Berl.×SL	225	77	302	0.040
Berl.×SP	208	54	262	2.692
Sc.L×SP	272	0	272	—
SL×Sc.L	252	0	252	—

The prickly form, SB, gave a clearly monohybrid segregation when crossed with Maik. (209 prickly : 70 non-prickly), but it has not been crossed with any of the *L. serriola* lines. There can, however, be little doubt that the same locus for spination is involved.

The following data show that monohybrid ratios with a slight, but consistent deficiency in the recessive class were found in crosses between the very prickly *L. serriola* line Sc.S.Afr. and four lines of cultivated lettuce, all non-prickly:

	Prickly	Non-prickly	Total	$\chi^2_{3:1}$	No. of families	$\chi^2_{het.\ fam.}$
Berl. × Sc.S.Afr.	397	112	509	2.437	6	7.569
Oakl. × „	230	73	303	0.133	4	6.053
Maik. × „	530	143	673	5.053	8	9.083
R.rouge × „	83	20	103	1.712	1	—
	1240	348	1588	8.064**		

$\chi^2_{het.(3)}$ for crosses = 1.271

In F_2 of a cross between Altaica and Sc.S.Afr., it was also possible to classify fairly accurately into two classes, one with spines on stem and midrib, the other with spines on the midrib only .The ratio between the two classes was found to be 196 : 62, which is a good fit for 3 : 1 ($\chi^2 = 0.129$).

The spination of Altaica was, however, found to be inherited in a different manner in other crosses. F_1-hybrids with *L. serriola* forms, had the spination of the *L. serriola* parent, and F_1-hybrids with *L. sativa* forms, had the spination typical of Altaica, but in the F_2-generations of both types of crosses, a continuous variation was found and it was not possible to classify on a two class basis. For such crosses a visual scale was adopted, ranging from 0 to 10, where 0 stands for complete absence of spines and 10 corresponds to the strong spination of Sc.S.Afr. For comparison, this scale was used also for some crosses between *L. sativa* lines and *L. serriola* lines, in which the segregation was clear cut. A strong correlation was found between the spination of the leaves and of the stem, and it was not necessary to use different scales for the different parts of the plant. Plants in class 6 and above, usually had spines also on the stem, whereas plants in class 5 and below, usually had spines only on the midribs of the leaves. Results from some F_2-generations, classified in this way, are presented in Table 16. The position of parent lines and F_1-hybrids on this scale has been indicated without giving the frequency of plants in different classes.

The data in Table 16 for the three crosses between *L. sativa* lines and Sc.H, show distributions with two different maxima or even discontinuos distributions, but in the crosses between the same *L. sativa* lines and Altaica and in the cross Altaica × Sc.H, the variation is continuous.

The density of spines on the midrib was ascertained in some of the material. The length of the midrib was measured on three basal leaves from each plant and the total number of spines counted. The average number of spines per cm midrib was calculated for each plant and the results are presented in Table 17. They show that the variation in

TABLE 16. *Inheritance of spination; visiual classification of F_2-generations.*

Crosses	Distribution of F_2-plants in spination classes											Total
	0	1	2	3	4	5	6	7	8	9	10	
	(*L. sativa* lines)					(Altaica)		(Sc. H)			(Sc. S. Afr.)	
Altaica×Sc.H				1	4	71	96	118				290
Berl.×Altaica	13	26	50	93	63	30						274
K.driv×Altaica	57	28	21	68	69	33						276
Asp.×Altaica	8	53	56	72	65	19						273
Berl.×Sc.H	33	5	6	6	4	5	106	74				239
K.driv×Sc.H	69	1	3	—	1	15	173	17				279
Asp.×Sc.H	40	6	5	3	—	5	118	50				227

TABLE 17. *Inheritance of spination; density of spines on the midrib.*

Parent lines or crosses	% plants with different numbers of spines per cm midrib												Number of plants
	1	2	3	4	5	6	7	8	9	10	11	12	
K.driv	90.0	10.0											10
Maik.	60.0	40.0											10
Altaica		10.0	60.0	30.0									10
SP			22.2	77.8									10
Sc.L			70.0	30.0									10
Sc.S.Afr.									40.0	20.0	30.0	10.0	10
F_2K.driv×Altaica	58.6	27.0	11.3	2.5	0.6								159
F_2Maik.×Sc.S.Afr.	31.9	—	—	2.1	19.2	14.9	8.5	6.4	2.1	2.1	4.3	2.1	6.4	47

spination is continuous in F_2 of K.driv×Altaica, but clearly discontinous in Maik.×Sc.S.Afr., which is in agreement with the results obtained by visual classification.

The data presented here suggest an interaction between modifying genes and four alleles for spination. As the designations S_1, S_2 etc. are reserved for self-incompatibility alleles, the allelic series for spination will be referred to as the *Sp*-series. It must be assumed that the spination of Altaica is due to an allele at the *Sp*-locus, otherwise the absence of non-prickly plants in crosses with *L. serriola* forms is difficult to explain. This allele, which will be called sp_1, has weak powers of manifestation and consequently the difference between the two expected classes is blurred in crosses with non-prickly *L. sativa* forms, which have the recessive allele *sp. L. serriola* forms with moderate spination, *e. g. Sc.H*, are assumed to have the allele sp_2 with stronger powers of manifestation than sp_1, but the difference between the two alleles is not sufficiently strong to give a clear cut segregation in F_2 in the presence of modifying genes. The very prickly form Sc.S.Afr., finally is assumed to have yet another and stronger allele, *Sp*, and the difference in powers of manifestation between this and sp_1 is sufficient to give a two class segregation in the cross Sc.S.Afr.×Altaica. No F_2-results are available from a cross between Sc.S.Afr. and moderately prickly forms, but F_1-hybrids of such crosses have the strong spination typical of Sc.S.Afr. The very large variation within the prickly class in the cross Maik.× Sc.S.Afr. (see Table 17) is evidence of the existence of modifying genes.

10. Male sterility

Plants which were absolutely pollen sterile, but produced a fair number of seeds after cross-pollination with normal plants, appeared in F_3 of the cross R.rouge×Sc.S.Afr. This pollen sterility was obviously under sporophytic control. The sterile plants were characterized by very narrow, sharply cut leaves and could therefore be recognized before the flowering stage. Typical specimens are illustrated in Fig. 35.

The course of events which leads to pollen abortion has not yet been thoroughly studied, but preliminary investigations have shown, that very few PMC are found in the anthers and that meiosis in these PMC is characterized by a high degree of asyndesis, nearly all the chromosomes being present as univalents at I M.

This type, which will be designated as MS, appeared in seven F_3-families which gave a total of 318 normal and 11 male steriles. This

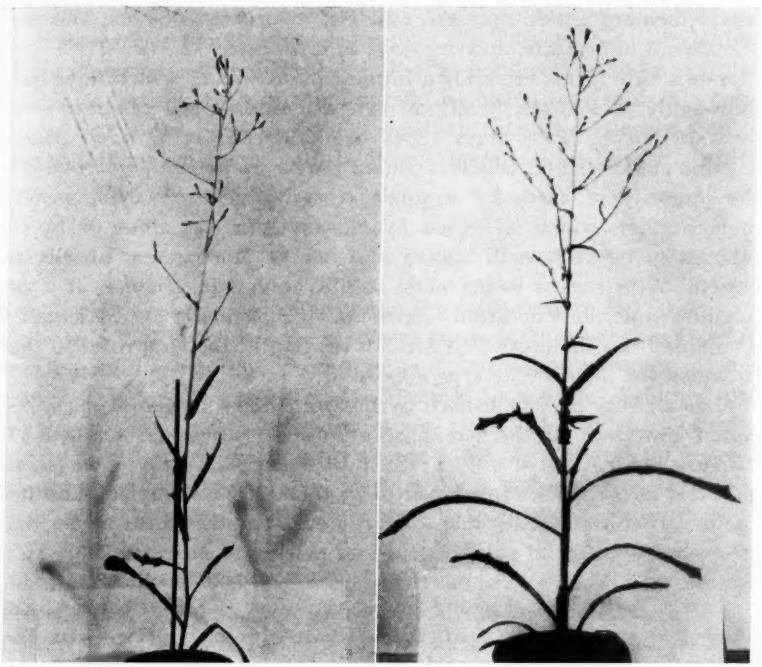


Fig. 35. Male sterile segregates from the cross R.rouge×Sc.S.Afr.

suggests dihybrid segregation in these families, although there is a shortage of male steriles, significant at the 5 % level ($\chi^2_{15:1}=4.743^*$). The F_3-families were grown in the field and the deficiency of the rather weak, narrow-leaved type is not surprising. No MS had been found in the F_2-generation which consisted of 109 plants. This, together with the fact that none of the F_3-families showed monohybrid segregation, indicates that at least three recessive genes are involved in the inheritance of the new type.

MS-plants were crossed with both parents. The F_1-hybrids were normal, and in F_2 the following segregations were recorded:

	Number of families	Normal	MS	Total	Segr.	$\chi^2_{dev.}$	$\chi^2_{het.}$
MS×Sc.S.Afr.	1	124	50	174	3 : 1	1.295	—
MS×R.rouge	2	432	24	456	15 : 1	0.758	6.590*

In spite of the heterogeneity of the data from the second cross these results provide strong evidence that the MS-type is due to three recessive

complementary genes, ms_1, ms_2 and ms_3, two of which, ms_2 and ms_3, are present in Sc.S.Afr. and one, ms_1, in R.rouge.

Male sterile forms are known in most plant species which have been thoroughly studied genetically. A review of this subject has been given by JAIN (1959). Two main types of male sterility are recognized: 1. genic male sterility, which is caused purely by the action of genes, in the majority of cases by a single recessive gene; 2. cytoplasmatic male sterility, which is caused by plasmatic factors alone or by the interaction between such factors and one or more genes, usually recessive. With regard to the male sterility found in *Lactuca*, it is not possible to decide whether it is genic or cytoplasmatic until results are available from the cross Sc.S.Afr.×R.rouge, *i. e.* the reciprocal to that, in which the male sterile type appeared.

Male sterility may be caused by the breakdown of one of many different processes in the chain of events of pollen development. In *Lactuca* the scarcity of PMC in the anthers suggests, that the degeneration sets in before the formation of mother cells is complete, and that the observed asyndesis is due to physiological disturbances rather than to a specific effect of the *ms* genes on pairing at meiosis. There is, at present, no information about the course of meiosis on the female side.

The association between male sterility and abnormal leaf shape is most probably a case of pleiotropy.

11. Seed colour

The variation and inheritance of seed colour in lettuce has been described and analyzed by DURST (1930) and THOMPSON (1943). Two major genes, *W* and *Y*, have been identified, and they interact to give the following colours of the seed coat:

WY: greyish brown to almost black; *Wyy*: yellow; *wwY* and *wwyy*: white. The segregation of these genes has been recorded in a number of crosses and the mode of inheritance found by DURST (*l. c.*) and THOMPSON (*l. c.*) has been confirmed. The segregation ratios for *W* and *Y* are summarized in Table 1.

12. Linkage

Only one case of linkage has previously been reported in lettuce, namely by THOMPSON (1938), who found that the anthocyanin genes *C* and *R* were linked, with a crossing-over value of 36 %. Anthocyanin is produced only if both the dominant genes *C* and *T* are present, and the *cc* class cannot be classified for *R* and *r*. Evidence of linkage is

therefore provided only by deviation from the monohybrid ratio of the *R*—*r* segregation among plants carrying *C*. In THOMPSON's material this segregation showed significant deviation in the direction to be expected in case of linkage, both in coupling and repulsion type crosses.

The two genes have segregated together only in one cross studied during this investigation, namely Wond. (*ccrr*) × Cont. (*CCRR*), which gave the following results:

				$\chi^2_{3:1}$	
CR	*Crr*	*cc*(*R*+*rr*)	*C*:*c*		*R*:*r*
171	73	90	0.675		3.148

The direction of the deviation in the *R*—*r* segregation is in this case opposite to that to be expected if it were due to linkage between *C* and *R*, and the results therefore do not support THOMPSON's finding. It has been observed that segregation at the *R*-locus i sometimes disturbed also in crosses in which no segregation occurs at the *C*-locus. This is shown by the fact that for the data presented in Table 1 the χ^2 for heterogeneity between crosses is significant at the 5 % level. In view of this, the linkage between *C* and *R* cannot be considered as established beyond doubt.

Although this investigation has not been planned specifically for linkage studies, data have been obtained from a large number of crosses were two or more genes segregated together. The material cannot be presented in full, but the analysis must be confined to cases of certain linkage.

V—*U*. — This linkage has already been noted in connection with the inheritance of lobing. The two genes segregated together in six crosses, two of the coupling type, four of the repulsion type:

Coupling	VU_{oak}	VU_{lob}	vU_{oak}	vU_{lob}	Total
Oakl. × Sc.S.Afr.	126	0	0	12	138
Sc.S.Afr. × Oakl.	104	0	0	8	112
	230	0	0	20	250

Repulsion	VU_{lob}	Vu	vU_{lob}	vu	Total
Sc.S.Afr. × Sc.H	262	136	116	0	514
Maik. × Sc.S.Afr.	284	198	81	0	563
Berl. × Sc.S.Afr.	92	99	26	0	217
Sc.S.Afr. × Maik.	46	46	14	0	106
	684	479	237	0	1400

This is obviously a case of very close linkage as no products of crossing over were identified either in coupling or in repulsion crosses.

Crossing over products have been sought in vain also in large F_2-generations of the crosses Sc.H×Sc.S.Afr. and R.rouge×Sc.S.Afr. Pleiotropy is unlikely, as the normal anthocyanin colour, which is produced by the dominant gene *V*, is associated in many lines of *L. serriola* with the kind of lobing produced by the gene U_{lob}.

The linkage group Lg—G—H—I. — The linkages *G—H* and *Lg—H* were referred to in connection with inheritance of hearting. It will be shown below, that the anthocyanin gene *I* belongs to the same linkage group. Dihybrid segregation data for the combinations *G—H*, *I—G*, *I—H*, *H—Lg* and *I—Lg* are presented in Table 18, together with an analysis by means of FISHER's scoring method. Data from crosses with partial pollen sterility in F_1 have been omitted from this table. The homogeneity of the data is, on the whole, satisfactory and the values of the recombination fractions indicate that the genes are arranged in the following way:

$$\longleftarrow\!\!\!

that factors affecting the segregation at these loci operate in some crosses. These abnormalities have not been analyzed thoroughly and their cause is not known. The slight deficiency of plants recessive for *w* and *st* is presumably due to viability differences and will not be considered further. The gene *V* is very closely linked to the *U*-locus, *i.e.* the locus of the alleles for lobing. The drastically disturbed segregation ratios observed for this locus, which are due to linkage with a gametophyte factor, *Ga*, will be discussed below. The genes *I*, *G* and *Lg* form a linkage group together with the hearting gene, *H*. It has been shown above that the abnormal segregation ratios for the genes of this linkage group are associated with partial pollen sterility of the F_1-hybrids. The nature of this pollen sterility and its connection with the disturbed segregation ratios will now be considered in detail.

Nature of the partial pollen sterility. — Partial sterility in intraspecific crosses is often due to structural chromosome differences, producing irregularities at meiosis in the F_1-hybrids. Many cases are, however, known of hybrids which have a more or less pronounced pollen sterility although the meiosis is perfectly regular. Thus BLAKESLEE (1928) found that in *Datura* certain lines behaved as 'bad pollen inducers', giving F_1-hybrids with up to 50 % pollen abortion, but only paired chromosomes at meiosis. MÜNTZING (1929, 1930 and 1932) found that in *Galeopsis* groups of pure lines could be distinguished, which were intra-fertile but inter-sterile, but that chromosome pairing was quite regular in fertile as well as in sterile hybrids. A similar situation was found by OKA (1957) in rice, and he explained these sterility phenomena by assuming that the parent lines differ with regard to gametic development genes, which he considered as duplicate genes that have originated through duplication of whole chromosomes. STEBBINS (1950), however, was of the opinion that sterility of this kind is due to structural differences, too small to have any visible effect on chromosome pairing at meiosis. He proposed the name 'cryptic structural hybridity' for this situation and he and STURTEVANT (1938) have suggested ways in which such minor structural differences may originate through inversions or translocations. MÜNTZING (1929, 1930) emphasized that the difference between interspecific and intraspecific sterility is a difference of degree only, and drew attention to the possibility that cases of intraspecific sterility are simple cases of the same nature as the more complicated sterility found in species hybrids, and that intraspecific sterility may be the consequence of interspecific hybridization in the past history of the species.

TABLE 18. *Dihybrid segregation data for the genes of the linkage group Lg—G—H—I.*

Crosses	Genes AB	AB	Ab	aB	ab	Total	Recombination fraction	D	I	χ^2
	G H									
Coupling:							0.2025±0.01382			
Berl.×Gr. 10		244	35	35	44	358		+48.7445	1695.4160	1.401
Berl.×Altaica		311	24	45	75	455		—73.9217	2154.7885	2.536
T+g×Rosette 48		199	27	28	38	292		+25.2924	1382.8533	0.423
		754	86	108	157	1105		+ 0.1152	5233.0578	4.360
	I G									
Coupling:							0.3302±0.01501			
Oakl.×Fl. 23		108	15	17	18	158		+35.0836	444.0745	2.772
T+g×IIGG		255	60	49	56	420		+41.8888	1180.4512	1.486
tttete×Fl. 23		42	15	11	7	75		—19.2916	210.7949	1.766
R.Liebl.×Sc.I		25	8	11	7	51		—11.5844	143.3405	0.936
R.Liebl.×V.grasse		88	20	17	15	140		+ 3.0358	393.4837	0.023
T+g×Rosette 48		183	44	43	22	292		—45.5720	820.6946	2.531
		701	162	148	125	1136				
Repulsion:										
Berl.×LC		191	70	80	6	347		+15.0335	449.8737	0.502
Berl.×Altaica		185	87	70	6	348		+22.1008	451.1702	1.083
iiGG×IIgg		155	53	48	11	267		—40.2990	346.1564	4.692
		531	210	198	23	962		+ 0.3955	4440.0397	15.791*
	I H									
Coupling:							0.2083±0.02142			
								19.4688	1342.9473	0.116

	531	210	198	23	962		+ 0.3955	4440.0397	15.791*

I H

Coupling:						0.2083 ± 0.02142		
T+g × Rosette 48	200	27	27	38	292		—12.4688	1342.9473	0.116
Repulsion:								
Laur. × Altaica	49	19	21	1	90		— 2.1739	100.1059	0.047
Berl. × Altaica	194	75	76	0	345		+26.2116	383.7391	1.790
Gr. 10 × Laur.	72	29	22	1	124		— 2.0723	137.9236	0.031
Maik. × Altaica	131	46	37	2	216		— 9.7691	215.6826[1]	0.443
	446	169	156	4	775		— 0.2725	2180.3984	2.427

H Lg

Coupling:						0.3476 ± 0.02236		
Maik. × Altaica	135	33	24	24	216		+16.6848	574.0946	0.485
Maik. × Rosette 48	179	28	47	28	282		+11.7176	623.5164[1]	0.220
Tezier × R.verte	179	33	61	29	302		—28.3871	802.6693	1.004
	493	94	132	81	800		— 0.0353	2000.2803	1.709

I Lg

Repulsion:						0.3912 ± 0.04499		
Maik. × Altaica	129	54	31	9	220		—17.1397	249.3562[1]	1.178
K.driv × Altaica	67	24	32	2	125		+17.1610	179.0679	1.645
	196	75	63	11	345		+ 0.0213	428.4241	2.823

[1] Adjusted for deficiency in one recessive class; *cf.* page 389.

In *Lactuca* seven lines, Asp., SL, SP, SB, Sc.H, Sc.S.Afr. and Sc.J behave, in BLAKESLEE's words, as 'bad pollen inducers'. There is no cytological evidence for the existence of any major structural differences between the 'bad pollen inducers' and other lines, and the partial pollen sterility must therefore be due to cryptic structural hybridity or mendelian gametic development genes.

There is reason to believe that the female fertility in hybrids within the *L. sativa-serriola* ecospecies depends not only on the genotypical constitution of the female gametes, but also on the development of the endosperm (LINDQVIST, 1960 a) which is determined by factors appertaining to the sporophyte, *i.e.* the partial female sterility sometimes observed is, in the terminology of MÜNTZING (1930), partly haplontic, partly diplontic. It is therefore to be expected, that the effect of gametic development factors should be less clear cut with regard to seed setting than with regard to pollen fertility and it is not surprising to find that the correlation between male and female fertility is poor (see data in Tables 2, 3, 5, 13 and 15, LINDQVIST, 1960 a). The further discussion will be confined to pollen sterility, as this reflects better than female sterility the effect of factors controlling the development of the gametophyte.

Connection between pollen abortion and disturbed segregation ratios. Genes which are linked with factors causing pollen abortion will, of course, show abnormal segregation ratios. In this connection it is immaterial whether the factors causing pollen sterility are mendelian genes or minute structural chromosome differences. As examples of such abnormal segregation ratios may be mentioned those described by MÜNTZING (1930, 1932) in *Galeopsis* and by OKA (1957) in rice. MÜNTZING found in one cross 50 % pollen sterility in F_1, and in F_2 abnormal segregations simulating linkage between two flower colour genes, which were inherited independently in other crosses. These results could be satisfactorily explained by assuming linkage between the flower colour genes and two lethal factors L and L_1. One of the parent lines was $LL\ l_1l_1$ and the other $ll\ L_1L_1$. In F_1, gametes of the constitution LL_1 and ll_1 aborted completely on the male side, but functioned to a certain extent on the female side. OKA (*l.c.*) explained the abnormal segregations found in rice, by assuming that the parental lines differed with regard to gametic development genes, one line carrying the genes X_1x_2 and the other the genes x_1X_2. In F_1, the combination x_1x_2 is lethal in the pollen, but the combination X_1X_2 is viable although it has a more or less reduced fertilizing capacity. According to this theory, one set of gametic

development genes can reduce the pollen fertility only to 75 % and several such mechanisms must be assumed, in order to account for the lower fertility observed in many crosses.

The disturbed segregations of the genes Lg, G, H and I. — It has been shown above with regard to the genes *Lg*, *G* and *I*, that the crosses showing abnormal segregation ratios differ significantly from each other with regard to the size of the deficiency in the recessive class. Thus the seven crosses showing disturbed ratios for the genes *G* and *g* can be divided into two groups, which are homogeneous *inter se* as shown below.

	G	*g*	Pollen fertility %
Berl.×Asp.	471	110	86.3
Berl.×SP	209	51	80.4
Berl.×SL	416	83	45.5
Asp.×Fl. 23	264	44	—
	1360	288	

$\chi^2_{5:1} = 0.777$
$\chi^2_{het.(3)} = 4.100$

	G	*g*	Pollen fertility %
Berl.×Sc.S.Afr.	340	42	67.4
Berl.×Sc.H	783	84	67.5
Sc.H×Fl. 23	337	37	—
	1460	163	

$\chi^2_{9:1} = 0.003$
$\chi^2_{het.(2)} = 0.514$

The much larger deficiency of recessives in the second group of crosses indicates that a larger proportion of pollen grains carrying *g* have been eliminated in this group than in the first. Neither the factorial scheme used by MÜNTZING nor that suggested by OKA can satisfactorily explain these results, but the following hypothesis appears the fit some of the facts.

The two yellow leaved lines Berl. and Fl. 23 are supposed to have a factor x_1 which is linked to the *G*-locus with a crossing over frequency of p %. Pollen grains with the factor x_1 are viable only in the presence of two other factors, X_2 and X_3, which are both present in Berl. and Fl. 23. The lines Asp., SP and SL have the genotype $X_1X_1x_2x_2X_3X_3$ and the lines Sc.S.Afr. and Sc.H have the genotype $X_1X_1x_2x_2x_3x_3$. F_1-hybrids of the crosses in the first group thus have the genotype $X_1x_1X_2x_2X_3X_3$ and F_1-hybrids of the crosses in the second group have the genotype $X_1x_1X_2x_2X_3x_3$. It can be shown that the output of male gametes of the F_1-hybrids in the first group will be $\frac{2-p}{3}G$ and $\frac{1+p}{3}g$ and in the second group $\frac{4-3p}{5}G$ and $\frac{1+3p}{5}g$. If it is assumed that female gametes carrying *G* and *g* function in equal proportions the expected segregations will

be, in the first group $\frac{5-p}{6}G$ *to* $\frac{1+p}{6}gg$ and in the second group $\frac{9-3p}{10}G$ and $\frac{1+3p}{10}gg$. The maximum likelihood method gives the following equation for the estimation of p:

$$L = 1360 \log \frac{5-p}{6} + 288 \log \frac{1+p}{6} + 1460 \log \frac{9-3p}{10} + 163 \frac{1+3p}{10}$$

Solution of this equation gives $p = 0.95 \pm 2.302$ %, *i.e.* a value of p which is not significantly different from 0. Test of agreement between the results of the two groups of crosses leads to a χ^2 for heterogeneity of only 0.589 (P=0.30—0.50). The results are thus satisfactorily explained on the above hypothesis. If the pollen abortion factor x_1 is absolutely linked to the *G*-locus, the expected segregation ratios are, in the first group 5 : 1 and the second group 9 : 1, which agrees extremely well with the observed ratios.

The pollen fertility of the F_1-hybrids is expected to be 75 % in the first group and 62.5 % in the second group. Agreement with the observed figures is reasonably good with the exception of the cross Berl.×SL, which has a pollen fertility much lower than that which is to be expected on the basis of the segregation *G—g*. It is possible that other factors for pollen abortion operate in this cross.

If a factor for pollen abortion, x_1, is very closely linked to the *G*-locus, it must be expected to show the same linkage relations as *G* to the other genes of the *Lg—G—H—I* linkage group. This can be tested by examining the segregation ratios of these genes in crosses in which one partner is a 'bad pollen inducer'. Such data are not available for the gene *H*, but data for *I* and *Lg* are presented below. Like the segregation data for *G—g*, they can, as shown below, be divided into two groups, one with a segregation ratio approaching 5 : 1 and another with a ratio approaching 9 : 1 and, as in the case of the *G—g* segregations, crosses involving the *L. serriola* lines Sc.H and Sc.S.Afr. have given the largest deficiencies of recessives.

	I	*i*	Total	$\chi^2_{5:1}$
SL×Altaica	173	36	209	0.047
Asp.×Fl. 23	184	45	229	1.468
	357	81	438	1.052

$\chi^2_{het.(1)} = 0.463$

	I	*i*	Total	$\chi^2_{9:1}$
Sc.H×Fl. 23	348	22	370	6.757*
Altaica×Sc.H	414	38	452	1.274
Sc.H×T+g	51	10	61	2.770
Sc.S.Afr.×Altaica	227	27	254	0.112
	1040	97	1137	2.725

$\chi^2_{het.(3)} = 8.188^*$

	Lg	*lg*	Total	$\chi^2_{5:1}$		*Lg*	*lg*	Total	$\chi^2_{9:1}$
Oakl.×Asp.[1]	227	60	287	3.714	Oakl.×Sc.S.Afr.[1]	277	26	303	0.678
SB×Maik.	226	54	280	1.383	Maik.×Sc.S.Afr.[1]	593	79	672	2.302
	453	114	567	4.829*		870	105	975	0.641
				$\chi^2_{het.(1)}=0.268$					$\chi^2_{het.(1)}=2.339$

[1] Including reciprocal crosses.

In spite of slight discrepancies revealed by the χ^2 test, these data appear to fit the hypothesis proposed above. The possible effect of pollen abortion genes on female fertility has been neglected in this discussion and the comparatively good agreement between expected and observed segregation ratios indicate, that this effect is small.

When crossing over values between X_1 and the other genes are considered, the agreement with the factorial scheme breaks down. The crossing over value for *Lg* and X_1, calculated as explained above for *G* and X_1, was found to be 4.58 ± 3.239 %. The arrangement of the genes of the linkage group *Lg—G—H—I*, shown on page 454 suggests for *Lg* and *G* a crossing over value of between 5 and 10 %. This does not differ significantly from the value found for *Lg* and X_1, but, on the other hand, 4.58 ± 3.239 is not significantly different from 0. The segregation data for *I—i* give for X_1 and *I* a negative crossing over value, $-3.85 \pm \pm 2.741$ %, which is not significantly different from 0. This does not agree at all with the value 33.02 ± 1.501 % which was obtained for *I* and *G* from the crosses in which both genes showed normal segregation. Thus *G* and X_1 show entirely different linkage relations, at least with *I*.

One explanation of this discrepancy suggests itself, namely that the pollen abortion factor x_1 does not represent a mendelian gene, but a structural chromosome difference which suppresses crossing over between *G* and *I*. This hypothesis demands that these two genes show absolute or very close linkage in crosses involving any of the lines which behave as 'bad pollen inducers', *i.e.* Asp., SL, SP, SB, Sc.S.Afr. and Sc.J. This is, however, not the case as shown by the following results:

Crosses	*IG*	*Ig*	*iG*	*ig*	Total
Asp.×Fl. 23	166	18	30	15	229
Sc.H×Fl. 23	318	29	17	8	372
	484	47	47	23	601

The crossing over value for *I* and *G*, calculated from these data with the product formula (IMMER, 1930) was found to be 28.39 ± 2.242 %, which is hardly significantly lower than that obtained from the crosses

in which the single gene ratios were normal $\left(\frac{d}{m}=1.72;\ P=0.05—0.10\right)$. Thus the factorial scheme suggested above does not fit all the facts, and the data at present available do not permit an explanation of the discrepancies.

The disturbed segregations at the U and V loci. — The abnormal segregation ratios at the *U* and *V* loci are not associated with any sterility in the F_1-hybrids and are therefore certation phenomena of the same nature as those described by such authors as MANGELSDORF and JONES (1926) in maize, SUTTON (1939) in *Tropaeolum*, BAENZIGER and GREENSHIELDS (1958) in *Melilotus* and BEMIS (1959) in lima beans. The hypothesis normally used to explain such aberrent segregation ratios is that advanced by MANGELSDORF and JONES (1926), that the genes showing disturbed segregation are linked with a gametophyte factor and that pollen grains carrying this factor have a rate of pollen tube growth inferior to that of pollen grains with the corresponding normal factor. If this reduced rate of pollen tube growth is due to a certain gametophyte factor, then the parent line carrying this factor must be expected to have a rate of pollen tube growth inferior to that of the other parent line. With regard to lobed and non-lobed forms of *Lactuca*, the only available evidence on this point has been contributed by MC COLLUM (1953), but it does not confirm this expectation. MC COLLUM made reciprocal crosses between a non-lobed form of *L. sativa* and two lobed forms of *L. serriola*, using a mass contamination technique. His results (which, however, require confirmation in the opinion of the author himself) indicate a much higher rate of growth of pollen tubes from *L. serriola* than from *L. sativa*. The F_2-data, however, confirm the results obtained by the writer and other authors in showing a deficiency of plants with the character introduced by the *L. serriola* parent, *i.e.* the lobed leaf character. This shows that gametes carrying the allele introduced by *L. serriola* had a fertilizing efficiency inferior to that of gametes with the allele from *L. sativa*.

An alternative explanation of the disturbed segregations at the *U*-locus is, that they are caused not by the effect of a single gametophyte factor, but by interaction of factors introduced into the F_1-hybrids by different parent lines. This is essentially the same explanation as that suggested above for the aberrant segregation ratios for the genes of the *Lg—G—H—I* linkage group, with the difference that the loss of fertilizing efficiency which leads to the abnormal segregation ratios for *U* and *V*, is not associated with visible deterioration of the pollen grains. This hypo-

thesis agrees with the view put forward by MÜNTZING (1930) that there is no difference in principal between certation phenomena, simple cases of intraspecific sterility and the more complicated sterility of species crosses, gradual transitions existing between the extremes.

2. Mutations at the St-locus

At the *St*-locus repeated mutations of the recessive to the dominant allele have been observed in the variety Tezier and in segregants from crosses with this variety, and there is evidence that the tendency to mutate is controlled by genes, possibly only one. Reversal from the recessive to the dominant condition takes place also with regard to hearting as found by the writer and by other authors (see pages 432 and 443). In the variety Kaiser Treib (syn. Kejsar driv) this reversal has been proved to be due to a mutation at the *K*-locus. The tendency to mutate from hearting to rosette habit is characteristic for certain lines, which suggests genotypic control also in this case. Further investigations are necessary in order to decide whether these cases of spontaneous mutations are entirely separate phenomena. It is, however, interesting, that in the variety Tezier, in addition to the mutation *st*→*St*, another mutation has been observed to take place repeatedly, giving non-hearting plants with a leaf shape similar to that of cos lettuce varieties. Whether the *K*-locus is involved in this change is not known.

Genes which mutate repeatedly, so called unstable genes, are known from many plant species. A review of such cases was made by DEMEREC (1935). Such mutations occur, as a rule, in the direction recessive→dominant and in the overwhelming majority of cases the reversal takes place in somatic tissue, giving rise to chimaeras. Cases are, however, known of plants with a dominant character appearing in the off-spring of plants, which had the corresponding recessive character and which did not show any signs of being chimaeras. An example is the dominant mutant '*crispa*' which regularly occurs in a certain strain of *Antirrhinum* with a frequency of about 2 % (BAUR, 1926). This case is similar to the reversal from striate vein to the normal dominant condition in *Lactuca* although with the difference that the '*crispa*' mutant was lethal in the homozygous state. DEMEREC (*l.c.*) did not consider *crispa* as a typical unstable gene, but was inclined to interpret the reversal phenomenon as due to crossing over between two partly overlapping inversions or to some similar mechanism involving a small structural chromosome difference. Meiosis has been examined in Tezier, the normal

veined mutant and the cos off-type (LINDQVIST, 1960 a). Certain irregularities were found, particularly in the cos off-type, but similar abnormalities were found in many other lines of cultivated lettuce and therefore no conclusions can be drawn from these observations. However, the possibility cannot be excluded, that the difference between the normal leaf type and the striate-vein type is due to a small structural difference without any major effect on meiosis.

3. Genetics of hearting

Evidence has been found in the present investigation for the existence of three major recessive genes for hearting, *k*, *h* and *ca* and a number of recessive modifying genes. It has been pointed out that these results are not difficult to reconcile with those obtained by DURST (1930) and LEWIS (1930), who both found quantitative inheritance of the hearting characteristic, or those obtained by BREMER and GRANA (1935) and PEARSON (1956), who both found monohybrid inheritance.

Cabbage lettuce owes its name to its similarity with the other heart forming vegetable, the cabbage, *Brassica oleracea*, var. *capitata*. The inheritance of hearting in *Brassica* has been studied by several authors with differing results. KRISTOFFERSSON (1924) found that hearting tendency was inherited in a quantitative manner, PEASE (1926) found that his results could be accounted for by the existence of two factors N_1 and N_2, only the double recessives, n_1n_1 n_2n_2, forming good hearts. ALLGAYER (1928), however, found that hearting is a dominant character, determined by one major gene and he explained the difference between his and PEASE's results as due to different methods of classification. MALINOWSKI (1929), finally, found that the capacity to form hearts depends on the interaction of three recessive genes. Although the results of the different investigations are somewhat contradictory, there is thus reason to believe that hearting in lettuce and in cabbage is inherited in the same manner. It is interesting that the genetic mechanism for this character may be the same in plants belonging to two very remotely related families of the plant kingdom.

It is also interesting that the primitive, possibly wild *L. sativa* form which was obtained under the name *L. altaica* FISCH. and MEY., has two of the recessive genes, *k* and *ca*, and that in this respect it agrees with certain cultivated varieties, whereas the lines of *L. serriola* which have been analyzed, appear to have three dominant genes. This has a bearing on the problem of the origin of cultivated lettuce, which will be discussed in another connection (LINDQVIST, 1960 c).

4. Alleles in L. sativa and L. serriola

The present knowledge about the distribution of alleles at 19 different loci in *L. serriola* and in the various forms of *L. sativa* is summarized in Table 19. All the genes included in this table have been referred to in this paper with the exception of *B* and *b*. These designations were introduced by WHITAKER and MC COLLUM (1954) for the characters shattering and non-shattering. The writer has for the same genes used the letters *Er* and *er* (LINDQVIST, 1956), but the designations *B* and *b* must have priority.

Table 19 is compiled not only on the results of genetical analyses, but also, in the case of genes, the effect of which is easily visible, on observations on living material and herbarium specimens and on descriptions and illustrations by other authors. It does not seem justified to give a complete account here, of these observations. Some of the more important facts have been dealt with in another paper (LINDQVIST, 1960 c).

Only at the *B*-locus is one allele, the recessive, consistently present in *L. sativa* and the other, the dominant, in *L. serriola* (WHITAKER and MC COLLUM, 1954; LINDQVIST, 1956). This fact provides a very reliable means of distinguishing between the two taxonomic units. Some recessive genes are common within *L. sativa* whereas only the dominant alleles are found in *L. serriola*. Such genes are *t*, *i*, *g*, *lg*, *l*, *k*, *h*, *ca* and *w*. Among these *t*, *i*, *k*, *ca* and *w* are found in primitive as well as in cultivated forms of *L. sativa*. More rare recessive genes are *c*, *st* and *y* which are present only in a few varieties of *L. sativa*, and *v* which has been found only in one line of *L. serriola*. With regard to the *Sp*-locus, the alleles *Sp* and sp_2, which produce a large number of prickles on stems and leaves, predominate in *L. serriola* and are common in the primitive forms of *L. sativa* whereas, not surprisingly, only the recessive allele, causing absence of prickles, is found in the cultivated forms. The allele sp_1 is found only in the primitive form Altaica. Information about the genetics of waxiness is scant, but the recessive gene *gl* appears to be present only in Altaica and possibly in some cultivated varieties.

At most of the loci where a difference exists between *L. serriola* and *L. sativa*, the dominant alleles are found in the former species and the recessive alleles in the latter. Exceptions to this rule are provided by the *R*, *P* and *U* loci. The alleles *R*, r'' and r' are widespread within *L. sativa*, at least *R* and r', but only the recessive *r* appears to exist in *L. serriola*. The dominant gene *P* for pointed leaf apex is found in most of the primitive and some cultivated forms of *L. sativa*, but not or only exceptionally in *L. serriola*. The dominant allele U_{lob} is not found in

TABLE 19. *Alleles in L. sativa and L. serriola.*

Locus	Allele	*L. sativa*					*L. serriola*
		Cabbage lettuce	Leaf lettuce	Cos lettuce	Aspargus lettuce	Primitive forms	
C	*C*	+	+	+	+	+	+
	c	+	−	−	−	−	−
T	*T*	+	+	+	+	+	+
	t	+	+	+	+	+	−?
R	*R*	+	+	+	?	+	−
	r″	+	+	−	−	−	−
	r′	+	+	+	?	+	−
	r	+	+	+	+	+	+
I	*I*	+	+	+	+	+	+
	i	+	−?	+	−	+	−
V	*V*	+	+	+	+	+	+
	v	−	−	−	−	−	+
G	*G*	+	+	+	+	+	+
	g	+	−	−	−	−	−
Lg	*Lg*	+	+	+	+	+	+
	lg	+	+	+?	−	−	−
U	U_{oak}	−	+	−	+	−	+?
	U_{lob}	−	−?	−	−	+	+
	u	+	+	+	+	+	+
P	*P*	−	+?	+?	+	+	+?
	p	+	+	+	+	+	+
St	*St*	+	+	+	+	+	+
	st	+	−	−	−	−	−
Gl	*Gl*	+	+	+	+	+	+
	gl	+?	+?	+?	−	+	−
L	*L*	+	+	+?	+	+	+
	l	+	+?	+	−?	−	−
K	*K*	−	+	−	+	+	+
	k	+	+	+	−	+	−
H	*H*	−	+	+	+	+	+
	h	+	−	−	−	−	−
Ca	*Ca*	−	−	−	−	+	+
	ca	+	+	+	+	+	−
Sp	*Sp*	−	−	−	−	+	+
	sp_2	−	−	−	−	+	+
	sp_1	−	−	−	−	+	−
	sp	+	+	+	+	+	+
B	*B*	−	−	−	−	−	+
	b	+	+	+	+	+	−
W	*W*	+	+?	+	+	+	+
	w	+	+	+	?	+	−
Y	*Y*	+	+	+	+	+	+
	y	+	−	−	−	−	−

+ indicates presence, — indicates absence.

L. serriola, except perhaps as a very rare exception, but is found in several cultivated forms, particularly in leaf lettuce varieties.

L. sativa and *L. serriola* differ also with regard to the hypothetical loci *Ga* and X_1, X_2 and X_3, which have not been included in Table 19. The existence of the *Ga* locus has been assumed in order to explain the abnormalities in the inheritance of lobing, and the fact that crosses between *L. sativa* and *L. serriola* usually give abnormal segregation ratios for this character indicates, that the allele typical for *L. serriola* is comparatively rare in *L. sativa*. The factors X_1, X_2 and X_3 were postulated in order to account for the occurrence of partial pollen sterility and abnormal segregation ratios for the genes *Lg*, *G*, *H* and *I*. Certain lines of *L. serriola* and primitive forms of *L. sativa* induced partial pollen sterility in F_1-hybrids when crossed with eight different lines from cultivated lettuce varieties, namely Berl., K.driv, Maik., Wond., Paris, R.rouge, G.W.Cos and Oakl. (see LINDQVIST, 1960 a, Tables 2 and 13), *i.e.* four cabbage, three cos and one leaf lettuce variety. This indicates that, according to the proposed hypothesis, most cultivated varieties have the factors x_1 X_2 X_3, but that various combinations of these factors exist in *L. serriola* and primitive forms of *L. sativa*.

The question of which conclusions may be drawn from these facts concerning the relationship between *L. sativa* and *L. serriola* and about the origin of cultivated lettuce, will be discussed in another paper (LINDQVIST, 1960 c).

SUMMARY

1. Analysis of genes controlling various characters has been undertaken with the following results:

Anthocyanin pigmentation. — Two new genes *I* and *V* have been identified; the recessive *i* intensifies, the recessive *v* reduces anthocyanin pigmentation. A new allele, r'', of the *R*-locus has been found.

Leaf colour. — The existence of two loci for leaf colour genes has been demonstrated. One of the genes is identical with BREMER's gene *G*, the other which is called *Lg* is presumably identical with THOMPSON's gene *G*. *Lg* and *G* are located on the same chromosome.

Leaf apex. — A dominant gene *P* produces pointed leaf apex, the recessive *p* gives blunt leaf apex.

Waxiness. — A recessive gene, *gl*, for almost complete absence of wax has been identified.

Photoperiodism. — Contrary to the results of BREMER and GRANA

(1935) the recessive gene *l* for insensitivity to photoperiod was found to be present only in cultivated lettuce.

Hearting. — This character was found to be controlled by three major genes, *K*, *H* and *Ca*, and a number of modifyers.

Spination. — Evidence has been found for the existence of an allelic series *Sp*, sp_3, sp_1 and *sp*.

Male sterility. — A case of male sterility determined by three recessive genes has been found.

2. The recessive gene, *st*, which gives the striate vein pattern, has in some lines a tendency to mutate to the dominant allele. The mutation takes place late in ontogeny and may be the result of minute structural rearrangements at meiosis.
3. Two linkage groups have been found, one consisting of the four genes *Lg*, *G*, *H* and *I*, the second of the very closely linked genes *U* and *V*.
4. Abnormal segregation ratios, associated with partial pollen sterility, have been found for the genes of the *Lg—G—H—I* linkage group. A hypothesis involving three complementary factors is proposed, to explain the abnormal ratios.
5. The aberrant segregation ratios at the *U* and *V* loci are explained as due to linkage with a gametophyte factor, but it is pointed out that complementary genes may be involved also in this case.
6. The distribution of alleles in *L. sativa* and *L. serriola* is summarized.

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ACTION COMBINÉE DU MYLERAN ET DES RAYONS X ET SON IMPORTANCE AU POINT DE VUE GÉNÉTIQUE

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A LA suite de recherches portant sur des systèmes biologiques divers tels que racines de *Vicia* (LOVELESS, 1953), d'*Allium* et de *Pisum* (TRUHAUT et DEYSSON, 1954), et cellules de tumeurs animales (TANAKA, OHNO, KINOSITA et BIERMAN, 1955), le Myleran fut utilisé en chimiothérapie anticancéreuse par HADDOW et TIMMIS (1953, 1956) ainsi que sur les cellules sanguines de rat par ELSON (1957). On connaît depuis lors son emploi comme agent antileucémique.

Par ailleurs, les recherches effectuées par O. G. et M. FAHMY (1956) ont montré que, chez la *Drosophile*, cette substance peut exercer des effets génétiques très spécifiques. Diverses recherches ont été réalisées en matière de mutagenèse chez l'orge (WETTSTEIN, GUSTAFSSON et EHRENBERG, 1959).

Etudiant les lésions causées par le Myleran ainsi que leur évolution dans les méristèmes radiculaires de *Vicia faba*, nous avons montré que la majeure partie des cassures chromosomiques dues à ce toxique devait être localisées dans des régions du génome connues comme étant hétérochromatiques (J. et M. MOUTSCHEN-DAHMEN, 1958). La localisation des lésions rapprocherait donc l'effet du Myleran de celui d'autres toxiques notamment de l'hydrazide maléique pour lequel une localisation des cassures dans l'hétérochromatine a été décrite par DARLINGTON et MCLEISH (1951) et MCLEISH (1953, 1954), quoique le mode d'action de ces deux substances puisse être radicalement différent. L'analyse cytologique des effets du Myleran a révélé un taux extrêmement faible de ponts chromosomiques.

[1] Chargé de recherches au Fonds National belge de la Recherche Scientifique.

Nous avons été amené à conclure que la grosse majorité du matériel chromatique isolé à la suite de cassures chromosomiques était perdue et ne se réintégrait pas au patrimoine héréditaire. Cette conclusion suggérant une application possible des substances nucléotoxiques dans l'analyse des systèmes héréditaires aussi bien que dans la mutagenèse, nous a incité à aborder l'étude des effets combinés du Myleran et des rayons X.

Les résultats préliminaires concernant les lésions chromosomiques des deux agents utilisés en combinaison sont résumés dans cette note.

I. TECHNIQUES

Les graines de *Vicia faba* de la variété commerciale «Åkerböna Weibull», sont traitées sèches par le Myleran. Dans ces expériences, le Myleran II, 1-4 di(méthane-sulfonyloxy)butane, est utilisé à la dose de 20 mgr pour 100 cc d'eau distillée. Afin de faciliter sa dissolution qui est assez malaisée, on le broie d'abord fortement dans un mortier; la solution est agitée continuellement pendant toute la période de dissolution qui se fait à 35° C. Elle est employée extemporanément (10 graines pour 100 cc de solution). La durée du traitement sera précisée au cours de l'exposé.

Les irradiations sont réalisées après hydratation des graines pendant des temps équivalant aux temps de traitement par le Myleran dans les conditions suivantes: 175 kV; 15 mA; distance focale 12,5 cm; sans filtre; débit 200 r/min. environ; doses de 1 Kr à 3 Kr. Après traitement, les graines sont placées en boîtes de Pétri sur papier filtre humecté; la température oscille entre 20 et 22° . Les méristèmes radiculaires sont récoltés entre 30 et 150 heures après la mise en germination. C'est la période durant laquelle s'étendent généralement les trois premiers cycles mitotiques. Le matériel est fixé par la solution de Carnoy.

II. RÉSULTATS

1. Myleran avant irradiation

Dans ces expériences, le Myleran est utilisé pendant des périodes de traitement allant de 10 minutes à 6 heures se plaçant immédiatement avant irradiation. Pour le Myleran utilisé seul, nous avons démontré qu'un temps d'action de 3 heures produit un maximum de cassures chromosomiques (J. et M. MOUTSCHEN-DAHMEN, 1958). Dans les

TABLEAU 1. *Modification des taux de mitoses (exprimés en pourcentages des taux de mitoses témoins) et des taux de figures mitotiques perturbés pour les deux agents utilisés seuls et en combinaison (seconde mitose après traitement).*

Traitements	Taux de mitoses	% de métaph. perturbées	% d'anaph. perturbées	Nombre de fig. étudiées
Myleran 20 mgr/100 cc 3 heures	92,8±3,2	23,3±2,8	5,4±0,6	300
Rayons X 3000 r	96,8±3,1	65,1±6,2	66,2±5,1	300
Myleran 20 mgr/100 cc 3 heures et rayons X 3000 r	50,5±6,2	91 ±8,4	68,5±5,8	300

Le tableau I montre clairement que:
1) la combinaison des deux agents détermine une inhibition mitotique;
2) les effets des deux agents s'additionnent.

tableaux 1 et 2, nous avons rassemblé les résultats des effets des deux agents utilisés seuls et en combinaison.

Les lésions sont relevées lors de la seconde mitose consécutive à la mise en germination. L'apparition de micronoyaux prophasiques ainsi que l'atténuation des effets primaires ou physiologiques sont pris comme critères permettant de délimiter ce cycle mitotique.

Concernant les cassures chromosomiques observées en métaphase et en anaphase, les données du Tableau 2 confirment celles du Tableau 1.

Nous avons déjà observé qu'après l'action du Myleran employé seul, le taux de ponts chromosomiques est extrêmement faible, voire nul dans nos conditions expérimentales. La Fig. 1 montre que la combinaison des deux agents diminue considérablement le taux de ponts obtenus avec les rayons X seuls. On peut donc conclure que dans les conditions expérimentales définies, la genèse de ponts paraît fortement inhibée.

Si l'on considère, d'après les résultats précédents, que la combinaison des deux agents est caractérisée par des effets cumulatifs dans la production de cassures chromosomiques et des effets inhibiteurs de la formation de ponts chromosomiques, on peut relever que:

1) un temps de traitement de 10 minutes paraît insuffisant à produire ces effets;

TABLEAU 2. *Modification des taux de cassures chromosomiques en méta- et en anaphase* [1] *pour les deux agents utilisés seuls et en combinaison (seconde mitose après traitement).*

Traitements	Cassures chromos. métaphasiques (par cellule)	Cassures chromos. anaphasiques (par cellule)	Nombre de fig. étudiées
Myleran 20 mgr/100 cc 3 heures	0,356	0,100	500
Rayons X 3000 r	1,133	0,793	500
Myleran 20 mgr/100 cc 3 heures et rayons X 3000 r	1,490	1,350	500

[1] En métaphase, cassures chromatidiques et isochromatidiques ont été rassemblées.

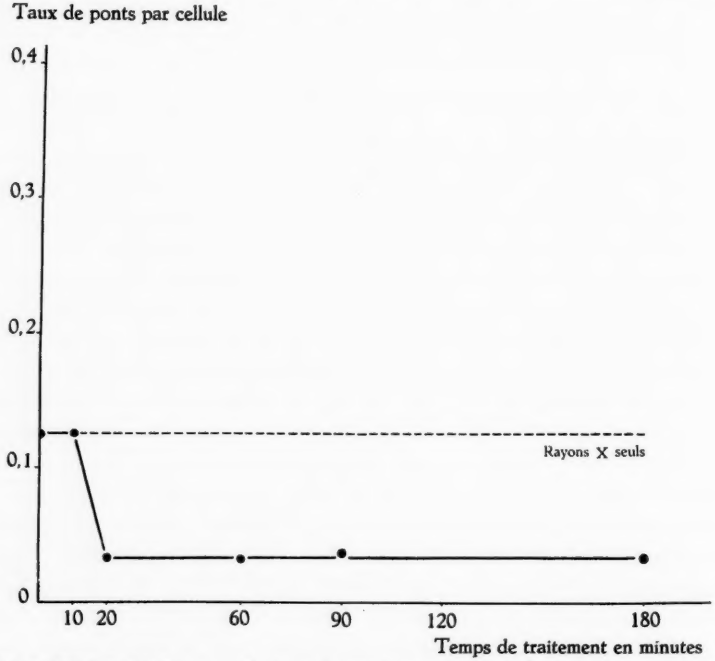

Fig. 1. Effet du Myleran sur le taux de ponts par cellule induit par les rayons X (en pointillé, taux de ponts induits par 1000 r.).

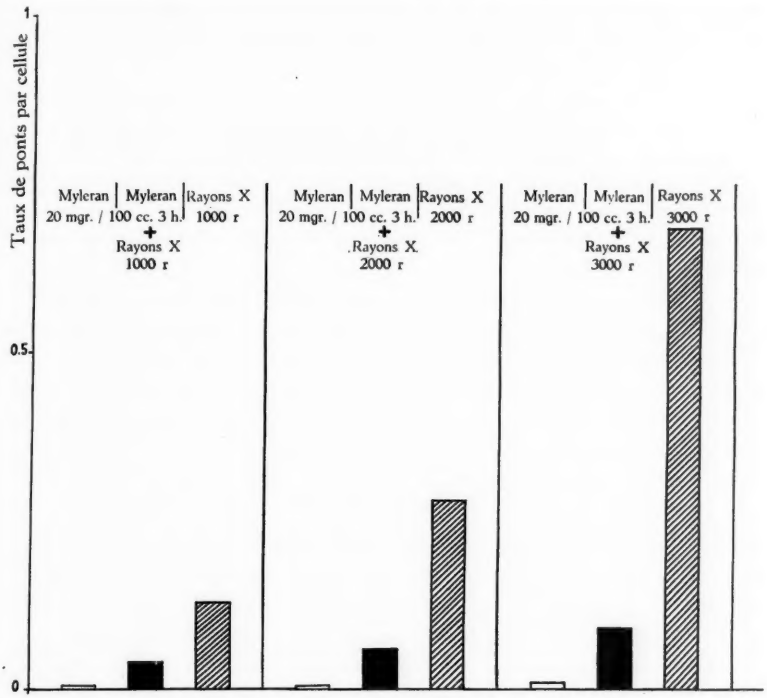
Taux de ponts par cellule
1
0.5
0
Myleran 20 mgr. / 100 cc. 3 h.
Myleran + Rayons X 1000 r
Rayons X 1000 r
Myleran 20 mgr. / 100 cc. 3 h.
Myleran + Rayons X 2000 r
Rayons X 2000 r
Myleran 20 mgr. / 100 cc. 3 h.
Myleran + Rayons X 3000 r
Rayons X 3000 r

Fig. 2. Effet du Myleran sur le taux de ponts par cellule pour des doses de 1000, 2000 et 3000 r. Dans chaque cas: à gauche, Myleran seul; à droite, rayons X seuls; au centre, les deux agents combinés.

2) un temps de traitement de 20 minutes suffit généralement à produire des effets;
3) pour des temps de traitement plus prolongés, le taux de cassures est accru par rapport au temps de traitement de 20 minutes mais il reste constant pour des temps de traitement plus longs que 90 minutes. Le taux de ponts reste le même quel que soit le temps de traitement.

La Fig. 2 résume l'effet du Myleran avant irradiation par des doses de 1000, 2000 et 3000 r sur le taux de ponts par cellule (pour 500 cellules). Ce graphique montre que le taux de ponts après traitement par les deux agents en combinaison est significativement inférieur au taux de ponts observé après l'action des rayons X seuls. L'effet est

d'autant plus marqué que la dose de rayons X est élevée. Les ponts observés après l'action du Myleran seul consistent presqu'exclusivement en ponts dits de «stickiness».

2. Myleran après irradiation

Dans une autre série d'expériences, le Myleran a été utilisé après irradiation (dose de 3 000 r). Les deux traitements successifs par les rayons X et le toxique ont été séparés par des intervalles croissants allant de 0 à 30 heures.

Des résultats assez comparables aux précédents ont été retrouvés, concernant le taux de ponts et le taux de cassures. Néanmoins, ni l'effet inhibiteur des ponts, ni l'augmentation des taux de cassures ne s'avèrent si élevés que dans les expériences précédentes (§ 1) où le Myleran était utilisé avant irradiation. Nous avons observé un accroissement constant des taux de cassures pour des intervalles allant de 12 à 16 heures entre les deux traitements. Comme il est actuellement admis (WOLFF, 1957) que la période pendant laquelle les cassures restent «ouvertes» est de l'ordre d'une demi-heure avec possibilité d'une large variation suivant les conditions expérimentales, aucune explication n'a pu être donnée. De nouvelles expériences utilisant des intervalles de temps plus courts sont en voie de réalisation.

3. Modifications dans la répartition des cassures chromosomiques

Nous avons rappelé que les cassures dues au Myleran sont localisées dans les zones du génome considérées comme étant hétérochromatiques (J. et M. MOUTSCHEN-DAHMEN, 1958). Dans cette série d'expériences, la répartition des cassures dans le génome de *Vicia faba* a été étudiée lors de la seconde mitose consécutive au traitement. Quelques résultats sont résumés dans le Tableau 3.

Pour les deux agents utilisés en combinaison, il existe une proportion très forte de cassures non localisées dans les zones hétérochromatiques quoique les taux de cassures localisées dans ces mêmes zones reste élevé par rapport aux expériences avec les rayons X seuls.

Les rapports

$$\frac{\text{Nombre de cassures locallisées dans les chromosomes S acrocentriques}}{\text{Nombre de cassures localisées dans les chromosomes M métacentriques}}$$

sont voisins pour les expériences réalisées avec les rayons X et avec les deux agents.

TABLEAU 3. *Modifications dans la répartition des cassures chromosomiques dues à la combinaison des deux agents (seconde mitose après le traitement).*

Traitements	% localisé dans le chromos. M	% localisé dans le chromos. S	% localisé dans les zones hétérochrom.	Nombre de fig. étudiées
Myleran 20 mgr/100 cc pendant 3 heures	49,6	50,4	96,6	500
Rayons X 1000 r	28,6	71,4	5	500
Myleran 20 mgr/100 cc et Rayons X 1000 r	31,2	68,8	22	500

III. DISCUSSION

Les données concernant l'action combinée des deux agents doivent être interprétées à la lumière de nos connaissances concernant l'action de chaque agent. En utilisant les deux agents en combinaison, on voit que le taux de ponts chromosomiques est toujours plus élevé qu'avec le Myleran seul. Il est possible qu'un certain nombre de cellules puisse échapper à l'action du Myleran mais non à celle des rayons X. L'accroissement du taux de ponts pour des intervalles de 16 heures au moins entre irradiation et traitement par le toxique pourrait peut-être correspondre à la fin de la première moitié de l'interphase, moment où l'action des radiations se révèle. Il serait souhaitable dans l'avenir d'avoir des données sur tous les types de réunions possibles après l'action combinée des deux agents.

Nous avons émis l'hypothèse que, vu la localisation élective des cassures dues au Myleran dans l'hétérochromatine, le nombre restreint de réunions pouvait être imputé à des propriétés spéciales de cette matière chromosomique. L'effet combiné des deux agents nous montre qu'il n'en est rien puisque la majeure partie des réunions consécutives à l'action des rayons X peut être inhibée par le Myleran dans certaines conditions.

La conséquence des effets combinés pour la génétique est évidente. On pourrait disposer là d'un moyen très efficace pour induire des déficiences chromosomiques sous réserve que des lésions trop nombreuses

et trop profondes soient éliminées au cours du développement de la plante et se révèlent dès lors d'une moindre valeur en matière de mutagenèse.

Cependant, GUSTAFSSON et TEDIN (1954), ainsi que VON WETTSTEIN, GUSTAFSSON et EHRENBERG (1959) ont largement souligné l'importance des substances chimiques et des radiations en tant que moyens d'obtention de nouveaux systèmes d'équilibre chromosomique qui, sans être nécessairement des mutants, pourraient s'avérer intéressants en vue de l'amélioration des plantes. GUSTAFSSON et TEDIN considèrent que les mutations artificielles impliquent nécessairement une augmentation de variabilité du génotype.

Un autre avantage résultant de l'action combinée des deux agents pourrait être l'élimination de la difficulté que présente l'effet de position dans l'analyse génétique. Quoi qu'il en soit, nous pensons qu'en raison de la sélection active des lésions chromosomiques, de grandes réserves doivent encore être émises actuellement quant à l'applicabilité de ce procédé en matière de mutagenèse.

CONCLUSIONS

Lorsque le Myleran est utilisé en combinaison avec les rayons X avant et après irradiation:

1) les taux de cassures chromosomiques s'additionnent. Cet effet est moins marqué lorsque le Myleran est utilisé après l'irradiation.
2) la genèse de ponts chromosomiques est très fortement inhibée lorsque le Myleran est utilisé avant irradiation. Cette inhibition existe lorsque le traitement est effectué après irradiation mais il est, dans ce cas, davantage marqué lorsque le délai séparant les traitements par les deux agents n'excède pas 16 heures.
3) le Myleran ne modifie guère la répartition des cassures chromosomiques dues aux rayons X. Il y a néanmoins plus de cassures localisées dans les zones hétérochromatiques que lorsque les rayons X sont employés seuls.
4) ces conclusions laissent prévoir l'utilisation des effets des deux agents combinés en matière de mutagenèse.

Ces recherches ont été en grande partie réalisées au département de génétique de l'Institut de recherches forestières de Stockholm. Nous nous faisons un devoir et un plaisir d'exprimer toute notre gratitude à Monsieur le Professeur Å. GUSTAFSSON dont les conseils éclairés et les encouragements incessants de loin comme de près furent une aide indispensable à la réalisation de nos plans. Nous remercions vivement

Monsieur le Dr L. EHRENBERG pour ses suggestions nombreuses et fructueuses en matière d'action des toxiques. Nos remerciements vont aussi au Professeur A. HADDOW (The Chester Beatty Research Institute, London) qui a mis gracieusement à notre disposition une grande quantité de substance nécessaire aux expériences.

SUMMARY

Combined action of Myleran and X-rays on seeds of Vicia faba

Seeds treated with Myleran (1 : 4-dimethanesulphonyloxybutane) before and after irradiation with X-rays show a considerably modified effect on chromosome breakage. Twenty minutes of immersion in a solution containing 20 mgr per 100 cc of Myleran inhibit the majority of chromosome bridges observed after X-raying. Treatment of seeds after irradiation modifies the effect of X-rays on chromosomes according to the time when the compound was given. If seeds are treated 16 hours following irradiation, the average number of chromosome bridges is considerably increased but never reaches the level obtained with X-rays alone at the same dosage. Modifications of chromosome break distribution have been observed and some applications of this combined action of mutagenic agents in the field of cytogenetics have been stressed.

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QUANTITATIVE INHERITANCE AND DOMINANCE

I. THE COEFFICIENT OF RELATIONSHIP CAUSED BY DOMINANCE

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I. INTRODUCTION

THE relationship between individuals of diploid organisms that is caused by the effects of single genes (genic or additively genetic effects) has been expressed in two different ways. WRIGHT (1922), using his method of path coefficients, defined the coefficient of relationship between two individuals as the correlation between the two zygotes. MALÉCOT (1948), on the other hand, applied the laws of probability. He defined the "coefficient de parenté" between two individuals as the probability that a gene taken at random from any locus in one individual is identical by descent with a gene taken at random from the corresponding locus in the other individual. The coefficient of relationship as well as the "coefficient de parenté" is a function of the inbreeding coefficient (F), which is defined as the correlation between uniting gametes (WRIGHT, 1921, 1922) or the probability that the two genes of any pair in an individual are identical by descent (MALÉCOT, 1948).

Besides the relationship between individuals that is caused by the effects of single genes, there exists also a relationship between individuals that is caused by the effects of gene pairs (dominance effects). Corresponding coefficient of relationship caused by dominance is known only for relatives in a random mating population. FISHER (1918) evaluated this coefficient (by FISHER called the correlation between dominance deviations) for a number of different kinds of relatives in a random mating population. The same coefficients have also been derived by MALÉCOT (1948) and KEMPTHORNE (1957) by different methods. KEMPTHORNE (1957) defined his coefficient as the probability that the two genes at one locus of one individual are identical by descent with corresponding two genes of the other relative.

As regards the relationships between individuals caused by dominance in inbred populations no comprehensive study seems to have appeared.

In the present paper the coefficient of relationship between two individuals caused by dominance, in the following called the dominance coefficient of relationship or merely the dominance coefficient, will be evaluated for randomly bred as well as inbred populations. The dominance coefficient of relationship between two individuals is defined as the probability that the gene pair at any locus in one individual is identical by descent with the corresponding gene pair in the other relative (*cf.* KEMPTHORNE, 1957).

II. GENERAL FORMULA

Consider two individuals, X and Y, X having the genes a (from his sire, A) and b (from his dam, B) at one locus, and Y having the genes c (from his sire, C) and d (from his dam, D) at corresponding locus (Fig. 1). The dominance coefficient of relationship between X and Y (D_{XY}) is then

(1 a) $$D_{XY}=P\ (ab=cd).$$

For evaluation purposes P (ab=cd) can be written as

(1 b) $$P\ (ab=cd)=P\ (a=c)\ P\ (b=d \mid a=c)+P\ (a=d)\ P\ (b=c \mid a=d)-P\ (a=b=c=d).$$

The first two terms are self explanatory. The last term, P (a=b=c=d), has to be subtracted from the sum of the other two, as it would otherwise be counted twice, once in each of the first two terms.

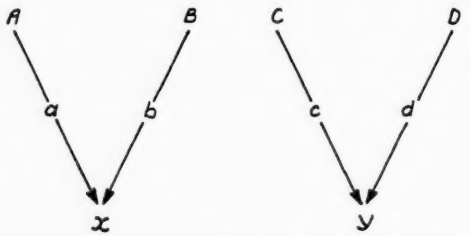

Fig. 1. Diagram showing two individuals X and Y, X having the genes *a* (from his sire, A) and *b* (from his dam, B) at one locus, and Y having the genes *c* (from his sire, C) and *d* (from his dam, D) at the corresponding locus.

It can be seen that, for a dominance coefficient of relationship to exist between X and Y, X's sire (A) must be related to Y's sire (C) as well as X's dam (B) to Y's dam (D) or X's sire (A) must be related to Y's dam (D) as well as X's dam (B) to Y's sire (C). In the language of path coefficients, a dominance coefficient of relationship between two individuals exists only if the two individuals are related through at least two chains of paths. These paths may pass through the same individual but must not at any place go through the same gamete.

In many instances it is very laborious to calculate P (ab=cd) in the form it is given above. Fortunately, it can often be simplified. In the following discussion different cases will be examined and the dominance coefficient of relationship given in as simple a form as possible. It is convenient to distinguish between the following three main cases: the two individuals are related 1) as ancestor and descendant, 2) in a collateral way, and 3) as ancestor and descendant as well as in a collateral way.

III. SIMPLIFIED FORMULAS

1. The two individuals related as ancestor and descendant

A. *The ancestor is not inbred.* Fig. 2 shows a simple general case, where the individual X is an ancestor to the individual Y. Y is related to X both through his sire (C) and his dam (D). If there are n generations between X and C and n′ generations between X and D the following is obtained:

$$P(a=c)\,P(b=d \mid a=c) = P(a=c)\,P(b=d) = \left(\frac{1}{2}\right)^{n+1}\left(\frac{1}{2}\right)^{n'+1} = \left(\frac{1}{2}\right)^{n+n'+2},$$

$$P(a=d)\,P(b=c \mid a=d) = P(a=d)\,P(b=c) = \left(\frac{1}{2}\right)^{n'+1}\left(\frac{1}{2}\right)^{n+1} = \left(\frac{1}{2}\right)^{n+n'+2}, \text{ and}$$

$P(a=b=c=d)=0$, as $F_X=0$ and $F_X=P(a=b)$ (MALÉCOT, 1948).

Thus

$$P(ab=cd) = 2\left(\frac{1}{2}\right)^{n+n'+2}$$

and

$$D_{XY} = \left(\frac{1}{2}\right)^{n+n'+1}. \tag{2 a}$$

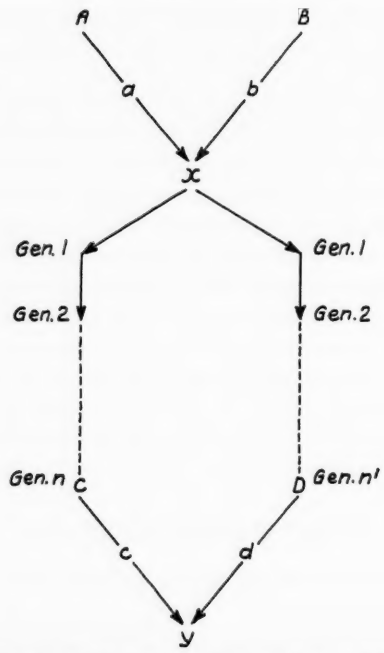

Fig. 2. Diagram showing X as an ancestor to Y, Y being related to X both through his sire (C) and his dam (D). C is n generations removed from X and D n′ generations. A and B are the parents of X. A transmits the gene *a* to X, B the gene *b* to X, C the gene *c* to Y, etc.

There is often more than one chain of paths connecting C with X and X with D. The formula then becomes

$$D_{XY} = \sum \left(\frac{1}{2}\right)^{n+n'+1}, \qquad (2\,b)$$

where the summation is over all connecting chains, as the chains are each an individual event and these events are mutually exclusive. The dominance coefficient of relationship between X and Y is thus equal to the inbreeding coefficient of Y with respect to X (WRIGHT, 1922), $F_{Y \cdot X}$.

It may happen that the path from C to X and the path from X to D pass through the same individual, Z. In this case the two paths are, as was mentioned on p. 483, allowed to pass through Z, as long as they do not pass through the same gamete.

B. *The ancestor is inbred.* If X has an inbreeding coefficient of F_X this means that P $(a \neq b) = 1 - F_X$ and P $(a=b) = F_X$ (MALÉCOT, 1948). The case $a \neq b$ has just been treated. When $a=b$ the following is obtained (*cf.* Fig. 2):

$$P\,(a=c)\,P\,(b=d \mid a=c) = P\,(a=c)\,P\,(b=d) = \left(\frac{1}{2}\right)^{n}\left(\frac{1}{2}\right)^{n'} = \left(\frac{1}{2}\right)^{n+n'},$$

$$P\,(a=d)\,P\,(b=c \mid a=d) = P\,(a=d)\,P\,(b=c) = \left(\frac{1}{2}\right)^{n'}\left(\frac{1}{2}\right)^{n} = \left(\frac{1}{2}\right)^{n+n'},$$

$$P\,(a=b=c=d) = \left(\frac{1}{2}\right)^{n+n'},$$

and thus

$$P\,(ab=cd) = \left(\frac{1}{2}\right)^{n+n'}.$$

The wanted P $(ab=cd)$ is then $=$

$$(1-F_X)\left(\frac{1}{2}\right)^{n+n'+1} + F_X\left(\frac{1}{2}\right)^{n+n'}$$

and thus

$$D_{XY} = \left(\frac{1}{2}\right)^{n+n'+1}(1+F_X). \tag{3 a}$$

The complete dominance coefficient of relationship between X and Y is thus

$$D_{XY} = \sum\left(\frac{1}{2}\right)^{n+n'+1}(1+F_X), \tag{3 b}$$

where the summation is over all connecting chains, or $F_{Y \cdot X}$. The coefficient for X and Y, X being an ancestor to Y, is thus equal to the inbreeding coefficient of Y with respect to X, irrespective of whether X is inbred or not.

When X is inbred a dominance coefficient of relationship between X and Y exists if there are at least two independent chains of paths connecting Y and Z, Z being an ancestor to Y and a descendant to X, but only one chain connecting Z and X. A simple general case of this type is outlined in Fig. 3. The number of generations between X and Z is n, the number between Z and C (Y's sire) n', and the number between Z and D (Y's dam) n''. If the inbreeding coefficient of X is F_X, the following is obtained:

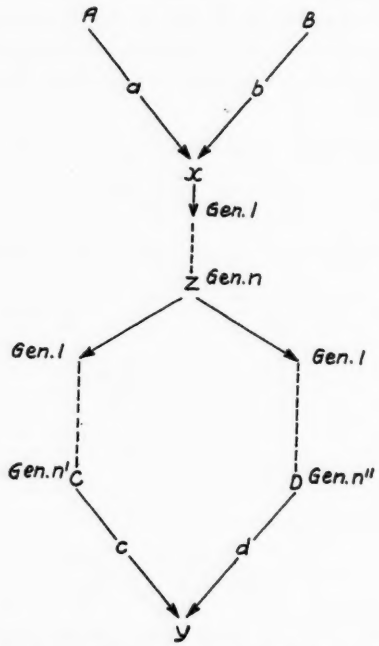

Fig. 3. Diagram showing X as an ancestor to Z and Y, Z as an ancestor to Y. Y is related to Z both through his sire (C) and dam (D). Z is related to X only through one of his parents. The number of generations between X and Z is n, between Z and C n′, and between Z and D n″. A transmits the gene *a* to X, B the gene *b* to X, C the gene *c* to Y, etc.

$$P\,(a=c)\;P\,(b=d \mid a=c)=F_X\left(\frac{1}{2}\right)^{n-1+n'+1}\left(\frac{1}{2}\right)^{n''+1}=F_X\left(\frac{1}{2}\right)^{n+n'+n''+1},$$

$$P\,(a=d)\;P\,(b=c \mid a=d)=F_X\left(\frac{1}{2}\right)^{n-1+n''+1}\left(\frac{1}{2}\right)^{n'+1}=F_X\left(\frac{1}{2}\right)^{n+n'+n''+1},$$

$$P\,(a=b=c=d)=F_X\left(\frac{1}{2}\right)^{n-1+n'+1+n''+1}=F_X\left(\frac{1}{2}\right)^{n+n'+n''+1},$$

and thus

(4 a) $$P\,(ab=cd)=D_{XY}=F_X\left(\frac{1}{2}\right)^{n+n'+n''+1}$$

or

(4 b) $$D_{XY}=F_X\sum\left(\frac{1}{2}\right)^{n}\sum\left(\frac{1}{2}\right)^{n'+n''+1},$$

where the last summation is over all chains connecting C with Z and Z with D, and the first summation is over all possible Z-individuals.

2. The two individuals related in a collateral way

A. *Regular inbreeding systems.* Methods similar to the ones used in Section 1 on ancestor-descendant cases may be applied to regular inbreeding systems in the earlier stages of inbreeding, when the dominance coefficient of relationship between two collateral relatives is wanted. With earlier stages of inbreeding is meant, as long as the events $a=c$ and $b=d$ as well as the events $a=d$ and $b=c$ are independent. This independence is lost with continued inbreeding, and when this stage has been reached the path diagrams are inadequate for the evaluation of the dominance coefficient.

However, if, depending upon the type of collaterals considered, the frequencies of the mating types of the parents or grandparents etc. are known, the dominance coefficient of relationship can be obtained. The coefficient is simply the sum of the frequencies of appropriate mating types after these frequencies have been multiplied by a figure that depends upon the type of collaterals considered, is characteristic of each mating type, and is easily estimated from the Mendelian laws.

The following example will serve to illustrate this point. The dominance coefficient of relationship between two full sibs produced by the selfing of an individual in the n^{th} selfing generation is wanted. The procedure to obtain this coefficient is then as follows. The generation equation for selfing is

$$\begin{pmatrix} v_n \\ w_n \end{pmatrix} = \begin{pmatrix} 1 & \frac{1}{2} \\ 0 & \frac{1}{2} \end{pmatrix} \begin{pmatrix} v_{n-1} \\ w_{n-1} \end{pmatrix},$$

where v_n is the frequency of homozygotes in generation n of selfing, w_n the frequency of heterozygotes in generation n, v_{n-1} the frequency of homozygotes in generation $n-1$, etc. After diagonalization of the generation matrix the following is obtained

$$v_n = v_0 + w_0 - \left(\frac{1}{2}\right)^n w_0 = 1 - \left(\frac{1}{2}\right)^n w_0$$

$$w_n = \left(\frac{1}{2}\right)^n w_0.$$

Each homozygote will leave offspring of only homozygotes, of the same type as the parent. Each heterozygote, on the other hand, will leave offspring of three kinds, one half heterozygotes of the same type as the parent, one quarter of one homozygous type and one quarter of another homozygous type. P (ab=cd) for two full sibs after a heterozygous parent is therefore $\frac{1}{2}\cdot\frac{1}{2}+\frac{1}{4}\cdot\frac{1}{4}\cdot 2=\frac{3}{8}$, and the wanted dominance coefficient of relationship

$$D_{XY}=v_n+\frac{3}{8}w_n=1-\frac{5}{8}\left(\frac{1}{2}\right)^n w_0. \tag{5 a}$$

If the inbreeding coefficient of the individual with which the inbreeding started is F_0, the dominance coefficient of relationship can be written as

$$D_{XY}=1-\frac{5}{8}\left(\frac{1}{2}\right)^n(1-F_0), \tag{5 b}$$

as $v_0=F_0$ and $w_0=1-F_0$ (MALÉCOT 1948).

It is of interest to note that the inbreeding coefficient of an individual in the n^{th} selfing generation

$$F_n=v_n=1-\left(\frac{1}{2}\right)^n(1-F_0). \tag{6}$$

The dominance coefficient of relationship between two full sibs produced by an individual in the n^{th} generation of selfing can therefore also be written in the form

$$D_{XY}=\frac{3}{8}+\frac{5}{8}F_n. \tag{5 c}$$

The generation matrix of full-sib mating has been studied extensively (HALDANE, 1937, 1955; FISHER, 1949; KEMPTHORNE, 1955, 1957). The frequency of any mating type in any generation can easily be written down from FISHER (1949, pp. 36 and 37). As the mating types in generation n are the same as the types of full-sib pairs in generation n, the dominance coefficient of relationship between two full sibs in generation n of brother-sister mating is

$$D_{XY}=t_n+v_n \tag{7 a}$$

in FISHER's (1949) notation, where t stands for the mating type aa×aa and v for ab×ab. If the inbreeding is started (generation 0) with two non-inbred, unrelated individuals

(7 b) $$D_{XY} = t_n + v_n = 1 - \frac{1}{2}\varepsilon^n - \frac{4}{5}\varepsilon^{n+1} - \frac{9}{10}(\varepsilon')^n + \frac{4}{5}\varepsilon(\varepsilon')^n + \frac{1}{5}\left(\frac{1}{2}\right)^n + \frac{1}{15}\left(\frac{1}{4}\right)^n + \frac{2}{15}\left(-\frac{1}{8}\right)^n,$$

where $\varepsilon = \frac{1}{4}(1+\sqrt{5})$ and $\varepsilon' = \frac{1}{4}(1-\sqrt{5})$ or $\frac{1}{2} - \varepsilon$.

The inbreeding coefficient of an individual in the n^{th} generation of full sibbing

(8 a) $$F_n = t_n + \frac{1}{2}u_n + w_n + \frac{1}{2}y_n,$$

where t denotes the mating type aa×aa, u the mating type aa×ab, w the mating type aa×bb, and y the mating type aa×bc.
Thus

(8 b) $$F_n = 1 - \frac{1}{5}\varepsilon^n - \frac{6}{5}\varepsilon^{n+1} - \frac{4}{5}(\varepsilon')^n + \frac{6}{5}\varepsilon(\varepsilon')^n.$$

Now F_{n-1} can be rewritten as

$$F_{n-1} = 1 - \frac{4}{5}\varepsilon^n - \frac{4}{5}\varepsilon^{n+1} - \frac{6}{5}(\varepsilon')^n + \frac{4}{5}\varepsilon(\varepsilon')^n.$$

It can then be seen that the dominance coefficient of relationship between two full sibs in generation n can be written as

(7 c) $$D_{XY} = \frac{3}{20} + \frac{3}{10}F_n + \frac{11}{20}F_{n-1} + \frac{1}{5}\left(\frac{1}{2}\right)^n + \frac{1}{15}\left(\frac{1}{4}\right)^n + \frac{2}{15}\left(-\frac{1}{8}\right)^n.$$

The following two paragraphs are included in order to establish the connections between the form in which F_n has been given above, and forms in which it has been given previously by other workers.

As $\varepsilon = \frac{1}{2} - \varepsilon'$,

$$F_n = 1 - \frac{1}{5}\varepsilon^n - \frac{6}{5}\varepsilon^{n+1} - \frac{1}{5}(\varepsilon')^n - \frac{6}{5}(\varepsilon')^{n+1}$$

or

(8 c) $$F_n = 1 - \frac{1}{5}S_n - \frac{6}{5}S_{n+1},$$

where $S_n = \varepsilon^n + (\varepsilon')^n$ and $S_{n+1} = \varepsilon^{n+1} + (\varepsilon')^{n+1}$.

This is the form, in which F_n was given by KEMPTHORNE (1955).

F_{n-2} can be written as

$$F_{n-2} = 1 + \frac{4}{5}\varepsilon^n - \frac{16}{5}\varepsilon^{n+1} - \frac{4}{5}(\varepsilon')^n + \frac{16}{5}\varepsilon(\varepsilon')^n.$$

It is then obvious that

$$F_n = \frac{1}{4} + \frac{1}{2}F_{n-1} + \frac{1}{4}F_{n-2}, \tag{8 d}$$

which is the classical formula obtained by WRIGHT (1921).

When more complex systems of inbreeding than full-sib mating are involved the generation matrix grows enormously. In brother-sister mating there are at most 4 alleles segregating. At most 5 alleles are segregating in a system of half-sib mating where in each generation one male is mated to two of his half sisters, which two half sisters are full sisters of each other. In such a system a 35×35 matrix is required if the dominance coefficient of relationship between any two collateral relatives is wanted. How large a generation matrix that is required for double-first-cousin matings, where 8 alleles have to be taken into consideration, cannot be said off-hand. But so much is certain that it is one far larger than 35×35 (the 12×12 matrix for double-cousin inbreeding given by FISHER (1949) is based upon the segregation of only two alleles).

In order to give some general idea how the dominance coefficient of relationship changes with inbreeding Fig. 4 has been included. The coefficient for two full sibs is there shown for selfing (formula 5 a, b or c), two kinds of parent-offspring mating (formulas 12 a or b and 13 a or b in the appendix), and brother-sister mating (formula 7 b or c), when the inbreeding is started with unrelated, non-inbred individuals. The dominance coefficient follows the same general pattern as the genic coefficient of relationship (WRIGHT, 1921). From Fig. 4 it is also evident that the type of parent-offspring mating, where mating is done to the younger parent in each generation, is not as effective as full-sib mating in raising the dominance coefficient of relationship. In additively genetic aspects these two systems of inbreeding are equal (JENNINGS, 1916; WRIGHT, 1921).

B. *Irregular inbreeding systems.* In most instances the general formula 1 b has to be used.

However, it may happen that the event (a=c) is independent of the event (b=d) as well as the event (a=d) of the event (b=c). If that is the case the general formula 1 b can be simplified to

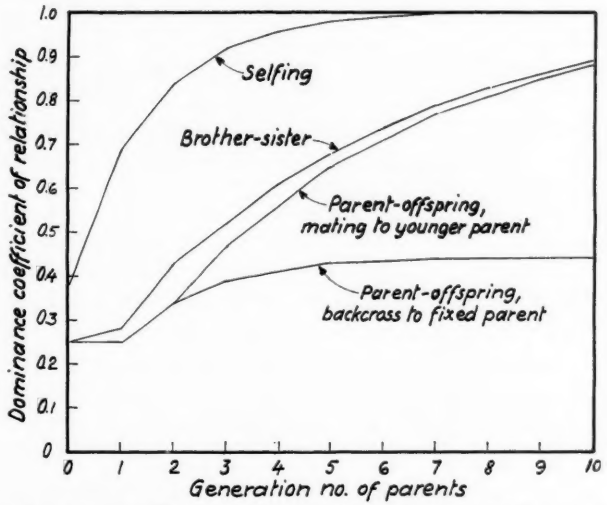

Fig. 4. The dominance coefficient of relationship between full sibs in successive generations under four systems of inbreeding, when the inbreeding is started with unrelated, non-inbred individuals (generation 0). For further details, see text!

(1 c) $P(ab=cd) = P(a=c)\,P(b=d) + P(a=d)\,P(b=c) - P(a=b=c=d)$.

Now $P(a=c)$ is the inbreeding coefficient of an individual that would theoretically result from a mating between A and C, the two sires (Fig. 1). This coefficient will be denoted by $F_{A\times C}$. Similarly, $P(b=d) = F_{B\times D}$, etc. In order for the event $(a=b=c=d)$ to occur all four genes must be derived from the same individual, P. This individual must therefore be an ancestor to all four of X's and Y's parents. Thus

$$P(a=b=c=d) = \Sigma F_{X\cdot P} F_{Y\cdot P},$$

where $F_{X\cdot P}$ is the inbreeding coefficient of X with respect to P, a common ancestor to A, B, C and D, etc., and the summation is over all possible P-individuals.

The dominance coefficient of relationship between X and Y is now

(9) $$D_{XY} = F_{A\times C} F_{B\times D} + F_{A\times D} F_{B\times C} - \Sigma F_{X\cdot P} F_{Y\cdot P}.$$

3. The two individuals related as ancestor and descendant as well as in a collateral way

A simple general case of this type is shown in Fig. 5. X is here an ancestor to Y through C, Y's sire (or dam). But X is also related col-

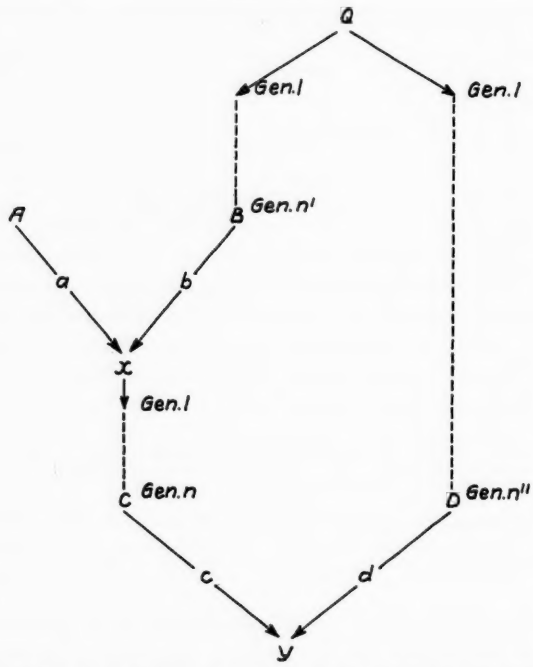

Fig. 5. Diagram showing X and Y related to each other as ancestor and descendant as well as in a collateral way. X's parents are A and B, Y's parents C and D. X is an ancestor to C, Q is an ancestor to B as well as D. The number of generations between X and C is n, that between Q and B n′, and that between Q and D n″. A transmits the gene *a* to X, B the gene *b* to X, C the gene *c* to Y, etc.

laterally to Y through Q, an ancestor common to B, X's dam (or sire), and D, Y's dam (or sire). The number of generations between X and C is n, between Q and B n′, and between Q and D n″. Furthermore, it is assumed that X has received the genes a and b from his parents A and B, respectively, and Y the genes c and d from his parents C and D, respectively. If Q is not inbred the following is obtained

$$P\,(a=c)\,P\,(b=d \mid a=c) = P\,(a=c)\,P\,(b=d) = \left(\frac{1}{2}\right)^{n+1} \cdot 2\left(\frac{1}{2}\right)^{n'+1+n''+1} = \left(\frac{1}{2}\right)^{n'+1+n+n''+1},$$

$$P\,(a=d) = 0$$

$$P\,(a=b=c=d) = 0,$$

and thus

(10 a) $P\ (ab=cd)=D_{XY}=\left(\frac{1}{2}\right)^{n'+1+n+n''+1}=F_{Y\cdot Q}$ (WRIGHT, 1922),

where $F_{Y\cdot Q}$ is the inbreeding coefficient of Y with respect to Q. If there are more than one individual of the same nature as Q,

(10 b) $$D_{XY}=\Sigma F_{Y\cdot Q},$$

where the summation is over all possible Q-individuals. These events can be added as they are mutually exclusive.

If Q is inbred, with an inbreeding coefficient of F_Q, the dominance coefficient of relationship between X and Y is derived as follows:

$$P\ (a=c)\ P\ (b=d \mid a=c)=P\ (a=c)\ P\ (b=d)=$$

$$\left(\frac{1}{2}\right)^{n+1}\left[(1-F_Q)\left(\frac{1}{2}\right)^{n'+n''+1}+F_Q\left(\frac{1}{2}\right)^{n'+n''}\right]=$$

$$\left(\frac{1}{2}\right)^{n'+1+n+n''+1}(1+F_Q)=F_{Y\cdot Q},$$

$$P\ (a=d)=0,$$

$$P\ (a=b=c=d)=0,$$

and thus

(11) $$P\ (ab=cd)=D_{XY}=F_{Y\cdot Q}$$

as before.

This result will be corroborated by an example from a regular inbreeding system. In the previous section it was dealt with full-sib mating (pp. 488—490). Using the notation of FISHER (1949) it can be seen that the dominance coefficient of relationship between a parent in generation n—1 and an offspring in generation n is

$$D_{XY}=t_{n-1}+\frac{1}{2}u_{n-1}+\frac{1}{2}v_{n-1}+\frac{1}{4}x_{n-1},$$

where x represents the mating type ab×ac (as regards the others, see pp. 488—489). After inversion of the generation matrix the mating type frequencies of generation n—1 can be expressed in terms of those of generation n. The dominance coefficient of relationship between parent and offspring then becomes.

$$D_{XY}=t_n+\frac{1}{2}u_n+w_n+\frac{1}{2}y_n=F_n \text{ (formula 8 a)},$$

which thus agrees with the result obtained above.

IV. DISCUSSION

A general formula (1 b) for the dominance coefficient of relationship between two individuals has been given. In many instances, however, this general formula can be simplified as shown in III. When the two individuals are related as ancestor and descendant the coefficient equals the inbreeding coefficient of the descendant with respct to that particular ancestor (formulas 2 b and 3 b). The dominance coefficient of relationship between two collateral relatives can often be expressed in terms of inbreeding coefficients (formulas 5 c, 7 c, 9, 12 b and 13 b). However, the coefficient for two full sibs under brother-sister mating contains all the latent roots of the generation matrix and not only those roots that are contained in the inbreeding coefficient (formulas 7 b and 8 b). When the two individuals are related as ancestor and descendant as well as in a collateral way the dominance coefficient of relationship equals the inbreeding coefficient of the descendant with respect to all ancestors that are common to the two individuals considered (formulas 10 b and 11). It is thus apparent that the dominance coefficient of relationship often is, but not always, a function of inbreeding coefficients.

The dominance coefficient of relationship was denoted u_{XY} by KEMPTHORNE (1957). He gave a formula for the genetic covariance of two individuals, X and Y, under random mating. This formula should be written as (where genic stands for additive or additively genetic):

$$\text{Cov}\,(X, Y) = \sum_{\substack{s=0,\,t=0 \\ s+t=1}}^{\substack{s+t=n \\ s=n,\,t=n}} (2r_{XY})^s\,(u_{XY})^t\,\sigma^2{}_G{}^s{}_D{}^t$$

where n = the number of loci involved,

r_{XY} = the "coefficient de parenté" (or $2r_{XY}$ = the coefficient of relationship),

$\sigma^2{}_G{}^s{}_D{}^t$ = the genic × genic × ... (s times) × dominance × dominance × × ... (t times) variance;
when s = 1 and t = 0, = the genic variance;
when s = 0 and t = 1, = the dominance variance.

In random mating populations therefore the dominance coefficient of relationship for a certain kind of relatives equals the correlation between the dominance deviations of relatives of that kind (*cf.* FISHER, 1918). Unfortunately, the formula cannot be extended to cover inbreed-

ing as well, as will be shown in a later paper. In inbred populations, however, as well as in randomly mated populations, the dominance coefficient of relationship has a value in itself in the same way as the coefficient of relationship or the "coefficient de parenté".

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I also want to express my gratitude to Dr. JAY L. LUSH for introducing me to the subject and for helpful criticism.

SUMMARY

The coefficient of relationship between two individuals caused by dominance is evaluated for randomly bred and inbred populations. This coefficient equals the probability that any gene pair in one individual is identical by descent with the gene pair at corresponding locus in the other individual.

A general formula valid for all cases is given. In many instances it can be simplified, as

1. The two individuals are related as ancestor and descendant: the dominance coefficient of relationship equals the inbreeding coefficient of the descendant with respect to that particular ancestor;

2. The two individuals are related collaterally: in regular inbreeding systems generation matrices furnish a help but otherwise the general formula or a slight modification of it has to be used;

3. The two individuals are related as ancestor and descendant as well as collaterally: the dominance coefficient of relationship equals the inbreeding coefficient of the descendant with respect to all ancestors common to the two individuals under consideration.

The dominance coefficient of relationship is therefore often, but not always, a function of inbreeding coefficients. It has a value in itself in the same way as the (genic) coefficient of relationship and the "coefficient de parenté". Moreover, in random mating populations it equals the correlation between dominance deviations.

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APPENDIX

The Coefficient of Relationship between Full Sibs Caused by Dominance in Two Systems of Parent-Offspring Mating

A. *Backcrossing to fixed parent.* If the inbreeding is started with two unrelated, non-inbred individuals, the dominance coefficient of relationship between two full sibs after the fixed parent and an individual in generation n of this type of parent-offspring mating is

(12 a) $$D_{XY} = \frac{7}{16} - \frac{3}{16}\left(\frac{1}{2}\right)^n$$

or

(12 b) $$D_{XY} = \frac{3}{16} + \frac{1}{2}F_n + \left(\frac{1}{2}\right)^{n+4}.$$

(Generation 0 is the generation in which the first mating between parent and offspring takes place.)

B. *Mating with younger parent.* If the inbreeding is started with unrelated, non-inbred individuals, the dominance coefficient of relationship between two full sibs after a mating between an individual in generation n of this type of parent-offspring mating and the younger parent of this individual is

(13 a) $$D_{XY} = 1 - \frac{1}{32}\varepsilon^n\left(13 + 31\frac{\sqrt{5}}{5}\right) - \frac{1}{32}(\varepsilon')^n\left(13 - 31\frac{\sqrt{5}}{5}\right) + \left(\frac{1}{2}\right)^{n+4}$$

or

(13 b) $$D_{XY} = \frac{3}{16} + \frac{1}{2}F_n + \frac{5}{16}F_{n-1} + \left(\frac{1}{2}\right)^{n+4}.$$

(Generation 0 is the generation in which the first mating between parent and offspring takes place.)

For the explanation of F_n, F_{n-1}, ε, and ε', see text!

A NEW MECHANISM FOR NUMERICAL INCREASE OF ACCESSORY CHROMOSOMES IN CREPIS PANNONICA

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IN addition to the 8 normal chromosomes of *Crepis pannonica* some plants of this species contain a number of accessory chromosomes (FRÖST and ÖSTERGREN, 1959). The study of meiosis in one such plant revealed a difference in the number of accessories between root tips and pollen mother cells. In order to test the validity of this observation, other plants were studied, and some crossing experiments were undertaken to investigate the mode of transmission of the accessory chromosomes.

I. CYTOLOGICAL OBSERVATIONS

The appearance of the ordinary chromosomes and of the accessory chromosomes is illustrated in Figs. 1 and 2 of FRÖST and ÖSTERGREN (1959). The accessories are rather small with a subterminal centromere and seem to be euchromatic. The chromosome number was determined in pollen mother cells and in root tips of six different plants. In all of them the root tips had only a single accessory chromosome, whereas pollen mother cells had two such chromosomes in addition to the 8 ordinary chromosomes. This difference was found constantly in all cells examined.

In order to decide whether the difference in number of accessories is characteristic of somatic cells *versus* germ line cells, or, as in *Xanthisma texanum* (BERGER and WITKUS, 1954), of root tips *versus* shoots, it would be necessary to examine various non-germinal tissues. So far, only a few observations have been made in anther tissues. Exact counts in tapetal cells showed that the number of accessories was doubled in some of them, just as in pollen mother cells (Fig. 1), whereas in other cells only a single accessory chromosome was found. Further studies are needed to reach a decision on this point.

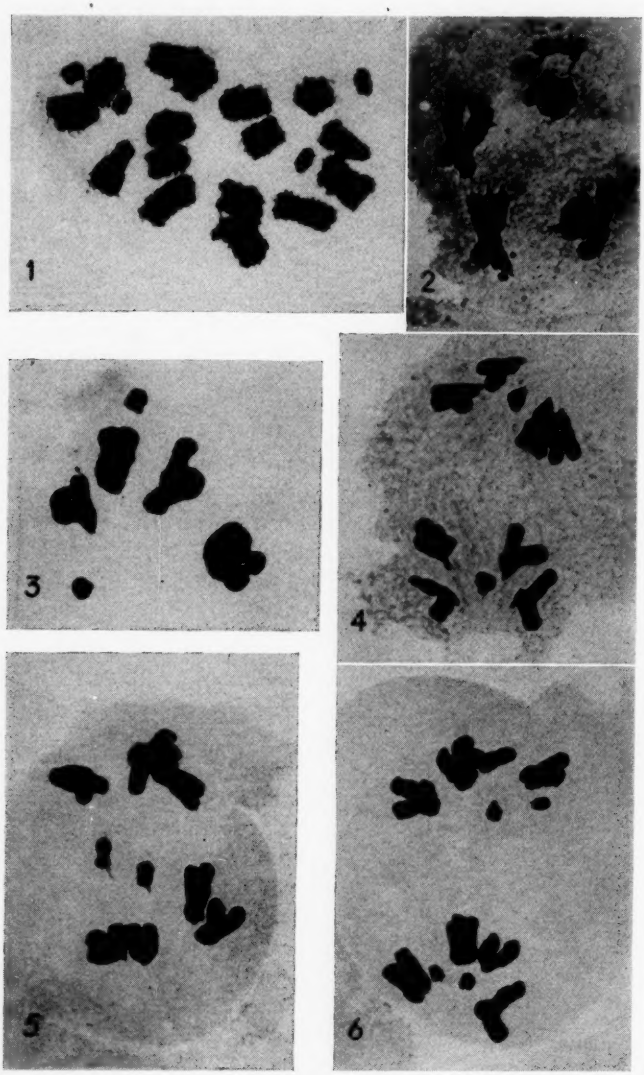

Fig. 1. Somatic metaphase in a tetraploid tapetal cell of *Crepis pannonica* showing four accessory chromosomes. In the root tips only one accessory chromosome was found. — Figs. 2—6. Different stages of meiosis in different plants with two accessory chromosomes in the pollen mother cells. All these plants had only one accessory chromosome in the root tips. — Fig. 2. Diakinesis with the two accessories

TABLE 1. *Inheritance of the accessory chromosomes in Crepis pannonica.*

Parents		F_1					
Plant no.	No. of acc. chr. root tips	No of acc. chr. (root tips)				No. of plants	$\bar{x}$ acc. chr. per plant
		0	1	2	3		
28—4×28—1	1×0	3	21			24	0.9
28—1×28—4	0×1		4			4	—
K3a—9×K3a—14	1×1		3	11	1	15	1.9

In the pollen mother cells of all six plants studied, the two accessories were regularly paired into a small bivalent (Fig. 2, FRÖST and ÖSTERGREN, 1959; Fig. 2, present paper). In about 25 per cent of the cells, however, the accessories were unpaired at metaphase I (Fig. 3) and oriented in or close to the equatorial region. The paired accessories separated regularly at anaphase along with the ordinary bivalents (Fig. 4). The univalent accessories, however, usually divided at anaphase I (Fig. 6), and lagging daughter univalents were often seen (Fig. 5). Micronuclei were seldom observed at telophase I, however, indicating that elimination of accessories is rare at the first division.

Most accessories were oriented on the equatorial plate at second metaphase and divided regularly at anaphase. As a result of the division at anaphase I of a certain fraction of accessories, single chromatids appear outside the plates at metaphase II. These chromatids are distributed more or less at random at second anaphase. Both vagrant accessories and micronuclei were observed in the tetrads, indicating a certain, but rather low, frequency of meiotic elimination of accessory chromosomes.

II. CROSSING EXPERIMENTS

The inheritance of the accessory chromosomes was studied in three cross combinations (Table 1). Seed setting in one of these crosses, viz. the combination 0×1 accessory chromosome, was poor. In eight other cross combinations attempted, no seeds were obtained, presumably be-

forming a bivalent. Fig. 3. Metaphase I with unpaired accessories. Fig. 4. Anaphase I showing regular separation of the accessory chromosomes which had formed a bivalent at metaphase I. Fig. 5. Anaphase I with lagging univalent accessories preparing for division. Fig. 6. Anaphase I showing divided accessory daughter univalents at the poles.

cause of incompatibility. The three progenies analyzed represented the following cross combinations:

(1) *Accessory chromosomes present only in the mother plant* (type of cross: 1×0). There is a marked increase, from parents to progeny, in average number of accessories in the root tips. Thus, it may be concluded that the embryosac mother cells of the maternal plant contain two accessories instead of the single accessory chromosome observed in the root tips. With normal meiotic behaviour of the two accessories, the maternal plant should mainly produce gametes with one accessory chromosome. In crosses of this type, therefore, most of the F_1 plants should have one accessory chromosome in their root tips. As seen in Table 1, this is the case, 21 out of the 24 F_1 plants having one accessory chromosome.

(2) *Accessory chromosomes present only in the pollen plant* (type of cross: 0×1). Only four F_1 plants were obtained. All four plants had one accessory chromosome in their root tips, as is expected when two accessories are present in the pollen mother cells of the paternal plant. Incidentally, one of the four F_1 plants was triploid and another was tetraploid. Since they both had a single accessory chromosome, they had probably originated from a diploid and a triploid egg cell, respectively, fertilized with ordinary haploid pollen.

(3) *Accessory chromosomes present in both parental plants* (type of cross: 1×1). In this case the parental plants were sister plants in the progeny of a cross of the type 1×0 accessory chromosomes. They were both expected to have germ cells with two accessory chromosomes, and accordingly gametes with one accessory chromosome should be mainly produced. Thus, most of the F_1 plants should have two accessory chromosomes in their root tips. Table 1 shows this to be the case. The few plants with one and three accessories are probably due to elimination or to irregular distribution of univalent accessories during meiosis.

The data so far obtained in the crossing experiments demonstrate clearly that an increase in number of the accessories takes place from generation to generation. In fact, the number of accessories in the progeny is on an average about 90 per cent higher than in the parents. The capacity for numerical increase is a characteristic feature of accessory chromosomes in general, and various mechanisms for attaining this have been observed in different materials. The mechanism active in *Crepis pannonica* is remarkably effective, since it is capable of inducing almost a doubling of the mean number of accessories from one generation to the next.

III. DISCUSSION

A difference in the number of accessories between germ cells and certain somatic tissues has been reported from several plant materials, as *Sorghum purpureo-sericeum*, *Poa alpina*, *Poa timoleontis* and *Xanthisma texanum* (JANAKI-AMMAL, 1940; DARLINGTON and THOMAS, 1941; MÜNTZING, 1946, 1948; NYGREN, 1957; BERGER and WITKUS, 1954; WITKUS, LOWERY and BERGER, 1955; BERGER, MCMAHON and WITKUS, 1955). In *Sorghum*, it sems that the accessories are eliminated from all somatic tissues and are retained only in the germ line. In *Poa alpina*, they are omitted from adventitious roots as well as in the leaves and are present only in the central parts of the plant and in the germ cells. In *Xanthisma texanum*, the accessories are eliminated from the roots but preserved in shoots and germ cells. Thus, in all these cases the variation in chromosome number is caused by a somatic elimination of the accessory chromosomes during various stages of differentiation, and, accordingly, the accessories are preserved only in specific tissues, always including the germ cells.

Whereas all earlier cases of variation in the number of accessories between somatic tissues and germ cells lead to the maintenance within the germ line of the same number of accessories as originally transmitted to the young zygote, the case of *Crepis pannonica* actually constitutes a mechanism for numerical increase of accessories from one generation to the subsequent one. The time and place of the process resulting in this doubling of the accessory chromosomes in the germ cells have not yet been traced. Most likely, a directed non-disjunction occurs at the time of differentiation of the shoot tissue, the increased number of accessories being restricted to areas destined to produce germ cells. As an alternative mechanism for the doubling, an endomitotic reduplication during the meiotic prophase may be considered. The accessory chromosomes on the female side of the turbellarian *Polycelis tenuis* seem to undergo a doubling of this type (MELANDER, 1950). In the present case, however, this process is less probable, and no evidence in favour of it has as yet been obtained.

In most cases, the numerical increase of accessory chromosomes takes place at or after meiosis. Thus, the well-known mechanisms active in various grasses involve directed non-disjunction during the first or second pollen mitoses. In *Secale cereale* there is a similar directed non-disjunction of accessories also at the first mitosis in the embryosac. In *Trillium* (RUTISHAUSER, 1956), *Lilium* (KAYANO, 1956, 1957) and *Plan-*

tago (FRÖST, 1959) numerical increase of accessories is effected by still another mechanism, viz. a preferential distribution of univalent accessories at meiosis of the embryosac mother cells toward those dyad cells that will form the egg cells. This mechanism seems to be more widespread than the preceding one, which has so far been found only in grasses.

The sequence of events leading to the doubling of the number of accessory chromosomes in the germ cells of *Crepis pannonica* will be studied further with the aim of ascertaining when and how the change in chromosome number arises.

SUMMARY

During a study of the accessory chromosomes of *Crepis pannonica*, a difference in their number was observed between root tips and pollen mother cells. Individuals with one accessory chromosome in the root tips constantly had two accessories in the pollen mother cells. As verified in crossing experiments, this difference was an expression of a new mechanism for numerical increase of accessory chromosomes, leading, very nearly, to their doubling from one generation to the next. The details as to when and how this doubling of the accessories is induced will be the object of further studies.

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THE MODE OF SEGREGATION IN A FAMILY OF HEXAPLOID *PHLEUM PRATENSE*

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THE present study has been made in order to analyse the segregation ratios of green and white seedlings of two successive generations of a family of the hexaploid *Phleum pratense*. Earlier investigations of *P. pratense* (MYERS, 1944; NORDENSKIÖLD, 1953, 1954) have demonstrated an autohexaploid mode of segregation in the investigated material, and the present study will give another illustration of this type of inheritance.

The plant selected for the present investigation belongs to the second isolated generation of an individual originating from Svalöf's Omnia, a commercial strain of timothy. It is a highly self-fertile plant and has given a simplex segregation after isolation, the ratio obtained being 2339 green : 825 white.

If a plant of a simplex constitution is selfed, two-thirds of the progeny plants are expected to be of the simplex type giving a segregation ratio of 3 : 1 and one-third of the duplex, giving a ratio of 24 : 1 (Table 1, category 1). However, earlier investigated individuals of the Omnia timothy have very often been found to contain several loci segregating for phenotypically similar chlorophyll deficiencies. Thus, the expected segregation ratios of the selfed progeny plants as well as the frequency of each segregation ratio in the progeny have also been calculated for individuals segregating for two independent loci (Table 1, categories 2—4). As the mother plant under investigation has given a simplex segregation ratio, at least one of the segregating loci is postulated to be of the simplex type. When we have two independently segregating loci, both of which are simplex, we shall obtain only simplex or duplex individuals, all of which are segregating for several loci (Table 1, category 2). Thus, the duplex individuals are all of the type segregating in

TABLE 1. *Expected per cent of plants with indicated segregation ratios in the selfed progeny of four hypothetical mother plants with genetic compositions stated below: 1) one locus segregating in simplex, 2) two loci independently segregating in simplex, 3) two loci independently segregating in simplex and duplex, 4) two loci independently segregating in simplex and triplex.*

(S=simplex, D=duplex, T=triplex)

Segregation ratios	1.28:1	2.57:1	2.97:1	3:1	11.8:1	22.6:1	24:1
1. Progeny of S:	—	—	—	66.7	—	—	33.3
2. Progeny of SS:	44.4	44.4	—	—	11.1	—	—
3. Progeny of SD:	16.7	38.9	16.7	2.8	15.3	8.3	1.3
4. Progeny of ST:	3.0	18.0	27.4	19.7	8.3	13.7	9.9

11.8 : 1 and none are expected to be of the one segregating in 24 : 1 (Table 1). If we have two independently segregating loci, one of which is simplex and the other duplex or triplex (Table 1, categories 3 and 4), we shall expect to obtain progeny plants of all possible types of simplex and duplex, giving the whole grading scale of segregation ratios stated in Table 1. The simplex progeny plants will have four ratios, ranging from the one of 1.28 : 1 to the one of 3 : 1. Among the duplex plants there are really two categories of segregation ratios, which can be recognized, one giving the ratios of 24 : 1 or 22.6 : 1, and the other giving the ratio of 11.8 : 1.

Thirty progeny plants, obtained after self-pollination of the studied mother plant, were investigated. The isolations were made during the summer of 1955, which was a very good summer for seed setting of timothy. The majority of the studied progeny plants were comparatively self-fertile, and 27 individuals gave seeds. The segregation ratios of green and white seedlings of the progeny plants are demonstrated in Table 2. From the table it is found that all the investigated plants gave both green and white seedlings: thus, no non-segregating plants were found.

Among the segregating plants in Table 2, 21 (nos. 1—21) are of the simplex and 6 (nos. 22—27) of the duplex type. Among the simplex plants there are, however, several which have given a large excess of white seedlings when compared with the ratio of 3 : 1. These do not give significance when compared with that ratio, but statistically fit the ratios of 2.57 : 1 or 1.28 : 1.

Among the six duplex plants the same phenomenon occurs. Here also only part of the plants give significance for the simple duplex segregation ratio of 24 : 1. One of the plants (no. 23) has a significantly proved

TABLE 2. *Numbers of green and white seedlings obtained after isolation of the investigated progeny plants. P-values from comparisons with some of the hypothetical segregation ratios expected in the progeny of an isolated individual segregating for two independent loci.*

Pl. no.	Number of seedl.		Green / White	P-values of indicated hypothetical ratios					Per cent white
	Green	White		1.28 : 1	2.57 : 1	3 : 1	11.8 : 1	24 : 1	
1	123	78	1.6 : 1	0.2 —0.1	< 0.001	< 0.001			38.8
2	47	27	1.7 : 1	0.3 —0.2	0.5—0.2	0.05—0.02			36.5
3	156	83	1.9 : 1	0.01—0.001	0.05—0.02	< 0.001			34.7
4	329	155	2.1 : 1	< 0.001	0.05—0.02	< 0.001			32.0
5	92	41	2.2 : 1		0.5—0.3	0.2—0.1			30.8
6	69	29	2.4 : 1		0.8—0.7	0.3—0.2			29.6
7	178	73	2.4 : 1		0.8—0.7	0.2—0.1			29.1
8	147	58	2.5 : 1		> 0.9	0.3—0.2			28.3
9	57	22	2.6 : 1		> 0.9	0.7—0.5			27.8
10	67	25	2.7 : 1		0.9—0.8	0.7—0.5			27.2
11	113	41	2.8 : 1		0.7—0.5	0.5—0.3			26.6
12	255	92	2.8 : 1		0.8—0.7	0.7—0.5			26.5
13	327	114	2.9 : 1		0.5—0.3	0.7—0.5			25.9
14	245	83	3.0 : 1		0.5—0.3	> 0.9			25.3
15	212	61	3.5 : 1		0.05—0.02	0.5—0.3			22.3
16	172	49	3.5 : 1		0.1—0.05	0.5—0.3			22.2
17	202	56	3.6 : 1		0.05—0.02	0.3—0.2			21.7
18	59	15	3.9 : 1		0.2—0.1	0.5—0.3			20.3
19	113	28	4.0 : 1		0.05—0.02	0.2—0.1			19.9
20	74	18	4.1 : 1		0.1—0.05	0.3—0.2			19.5
21	394	95	4.1 : 1		< 0.001	0.01—0.001			19.4
22	19	2	9.5 : 1						11.8
23	209	15	13.9 : 1				0.7—0.5	0.05—0.02	6.7
24	191	8	23.9 : 1				0.05—0.02	> 0.9	4.0
25	408	16	25.5 : 1				0.01—0.001	0.9—0.8	3.8
26	107	4	26.7 : 1				0.01—0.05	> 0.9	3.6
27	179	6	29.8 : 1				0.05—0.02	0.7—0.5	3.2

excess of white seedlings. This excess of white seedlings is most easily explained by postulating a segregation for white and green in two independent duplex loci, *i.e.* a segregation ratio of 11.8 : 1.

When comparing the obtained ratios of Table 2 with the calculated ones of Table 1 it is easily found that the only plausible constitutions for the mother plant are the simplex-duplex or the simplex-triplex ones (Table 1, categories 3 or 4). The mother plant cannot be presumed to segregate for only one simplex locus (Table 1, category 1) since the segregation ratios of several progeny plants (nos. 1—4 and 23) are explained solely by the postulate of two segregating loci. If the mother plant is postulated to be of the simplex constitution in two loci (Table 1, category 2), no duplex plants segregating for only one locus (24 : 1) would occur among the progeny plants. Thus, it would be impossible to explain the segregation ratios of the plants nos. 24—27. Both the two last alternatives of Table 1 presenting the expected ratios of a mother plant of the simplex-duplex or the simplex-triplex constitution give plausible explanations of the obtained segregation ratios in Table 2. They both give the whole gradual scale of segregation ratios found in the investigated material. To give a definite decision as to which of the two postulates is the most plausible is, however, impossible owing to the small amount of material available.

A graphic representation of the found and calculated values is given in the diagram (Fig. 1). Here the percentage of white seedlings of each individual plant of Table 2 has been plotted, beginning with the one with the highest percentage and ending with that with the lowest. In this way a "histogram" has been made, showing the gradual decrease of white seedlings in the studied material, and the proportions of plants with about the same segregation ratios. On account of the small material, it cannot be presumed, that all plants have been placed in absolutely the correct order, but it is only among the ones giving about the same percentage of white seedlings that an alteration in the order of the plants would be likely. Thus, the general appearance of the graph will probably not be changed, even if a larger material were to become available. In the same system the expected values of the progeny of a simplex-duplex and a simplex-triplex mother plant have been indicated, the former with a thin unbroken line and the latter with a thin broken one. The percentage of white seedlings have been stated according to the indicated segregation ratios of Table 2. The diagram shows a very close correspondance between the expected and the obtained values.

The mother plant of the investigated progeny has given 2339 green :

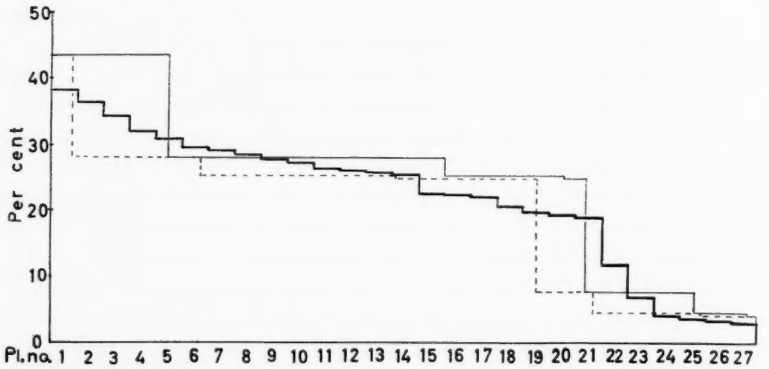

Fig. 1. Diagram showing the gradual decrease in number of white seedlings found in the studied material and expressed in percentage (the heavy line). In comparison are shown the expected values of two inbred progenies, the mother plants of which segregate in two independent loci, one simplex and one duplex (the thin unbroken line) or one simplex and one triplex (the thin broken line).

825 white seedlings. This ratio gives statistical fit for the ratios of 3 : 1 ($0.2>P>0.1$) or 2.97 : 1 ($0.3>P>0.2$), but in the comparison with the ratio of 2.57 : 1 there is a statistically proved deficiency of white seedlings ($0.02>P>0.01$).

From the comparison between obtained and expected ratios of both first and second isolated generations it is found that the most likely constitution of the mother plant is the one of two independently segregating loci, one occurring as simplex and the other as duplex or triplex. Whether the last-mentioned segregating locus is of the duplex or triplex type is impossible to prove with the present small material. From the graphs of Fig. 2 it is, however, found that the expected values of a simplex-duplex mother plant in general gives a certain deficiency of white seedlings. On the other hand, the postulate of a simplex-triplex mother plant, in many cases, gives an excess of white seedlings. This is especially the case for the progeny plants segregating for several loci in the expected ratios of 1.28 : 1 or 2.57 : 1. As a general under-representation of white seedlings seems more plausible than a general excess the first-mentioned postulate is supposed to be the more likely one, even though it cannot be statistically proved. Another phenomenon supporting this assumption is the fact that the white seedlings are not the only ones in the studied material representing segregating chlorophyll deficiencies. As has been shown for many other inbred plants of the Omnia timothy, genes for different kinds of yellowish chlorophyll deficiency

have also been observed. Moreover, plants containing many different genes for chlorophyll deficiencies are very often more likely to produce a deficit than to give an excess of recessives (GUSTAFSSON, 1940).

This analysis of the mode of segregation of a family of hexaploid *P. pratense* has been made in order to obtain further knowledge of the autohexaploid mode of segregation. The most obvious proof of an autopolyploid mode of segregation of the family is the fact that an individual, segregating in about 3 : 1, produces a progeny in which no non-segregating individuals are found. This phenomenon cannot occur if the study is dealing with a material having a disomic mode of segregation.

Another phenomenon that may be pointed out is the gradual scale of segregation ratios which are expected to occur in a family segregating as an autopolyploid for more than one locus. Many of the expected ratios are impossible to separate statistically for the individual plants. The diagram of Fig. 1 may serve as a concrete demonstration of the continuous variation of segregation ratios expected and obtained in an autopolyploid population.

SUMMARY

(1) The segregation ratios of green and white seedlings of a family of hexaploid *Phleum pratense* have been studied in two successive generations.

(2) The mother plant has a simplex segregation ratio of about 3 : 1 and all 27 investigated progeny plants segregated in green and white seedlings. This fact proves the autopolyploid mode of segregation of the family.

(3) From the segregation ratios obtained among the progeny plants (Table 2), the mother plant is postulated to segregate for green and white in two independent loci, one as simplex and the other as duplex or triplex.

(4) The continuous variation of the segregation ratio of the progeny of an autopolyploid individual segregating in more than one locus is demonstrated in Fig. 1, where the obtained and expected segregation ratios of the present study are compared.

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FREQUENCY OF MORPHOLOGICAL DEVIANTS AS A CRITERION OF DEVELOPMENTAL STABILITY

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I. INTRODUCTION

PHENOTYPIC manifestation of the genetic variation has been studied among others by TIMOFÉEFF-RESSOVSKY (1931), who states that the manifestation of a genetic difference is determined by three kinds of genes:

1. The main gene;
2. The genes which modify the intensity of the main gene (its penetrance and expressivity);
3. The genes which modify the specificity of the main gene.

Leaving aside the aspect of specificity, we may consider the concept of the intensity as it nears its limits in both directions; first with increasing intensity, which leads us to the genes with complete manifestation — the ideal tools of the geneticist; and secondly with main genes showing less and less intensity of phenotypic manifestation. This lower limit is interesting from the point of view of variation, for if the main gene has a very low manifestation then the phenotypic appearance of the trait will be dependent on the modifying genes of category 2 above, and will be sporadic and unpredictable. Its inheritance will be polygenic and influenced by interactions between the different unidentifiable genes that control the main switch gene. It is also possible that the switch function can be attributed to different genes in different genotypic environments, or even that no main gene is present but the switch mechanism is regulated by the attainment of a certain genotypic threshold value.

WADDINGTON (1957), in his survey of different kinds of natural selection, has distinguished three levels at which an adapted phenotype is guarded, and at which selection can act. What he calls *normalizing selection* restricts the genetic variation within a population by sorting

out the inferior extreme genotypes. It is equivalent to LERNER's term "stabilizing selection" and is based on the assumption of the superiority of intermediate phenotypes. The second kind of selection concerns the self-regulating capacity of the individual during development. WADDINGTON calls the selection which favours genotypes with a better canalisation, i.e. those which are less influenced by environmental stresses and minor variations in the genotype, *canalizing selection*. Its aim is to maintain "developmental homeostasis", and it seems clear that this quality is related to overdominance, in that heterozygotes often show less environmental variation than homozygotes. The third kind of selection is called by WADDINGTON *selection for repeatability*, and concerns the slight variations during development in a uniform environment which may cause asymmetry of homologous organs and slight deviations from normal adult appearance. Other authors have not distinguished between the last two kinds of selection, but WADDINGTON points out that this residual variation, which he calls "developmental noise", is to be regarded as the lowest limit of variation in *uniform* environment, and that its genetic control is not necessary identical with that for canalisation.

In a natural population the occurrence of abnormal morphological deviations is restricted by means of all these three kinds of selection. The frequency of a main gene can be reduced by normalizing selection; a buffering genotype can be built up by canalizing selection, and minor deviations from normal appearance can be kept infrequent by selection for repeatability.

Artificial selection in favour of deviants will give different results according to the genetic system that controls their appearance. If selection does not fix a special trait, the failure can depend on non-additive gene action or on incomplete penetrance of the main gene — or both.

Inbreeding may increase the frequency of deviants, since it can serve to fix certain epistatic combinations and to stabilize the genetic environment. Further, the increase in number of homozygous loci may lower the canalizing ability and thus increase the frequency of developmental accidents. It may also lead to that level of homozygocity which is necessary for the appearance of the special kind of deviants which LERNER (1954, 1958) has called *phenodeviants*. Thereby he means the sporadic, abnormal morphological deviations which appear after inbreeding, and the manifestation of which he claims is due to a certain level of homozygocity among the numerous loci that condition the trait.

The present investigation is another contribution to the data accu-

mulating on the effects of artificial selection and forced inbreeding. It deals with unspecific morphological deviants found in an ordinary wild type stock of *Drosophila melanogaster*. Different lines, originating from this stock, have been subjected to close inbreeding with and without selection for deviants, and also selection with less inbreeding. A comparison has also been made of different criteria of developmental stability to find out if they agree in their response to outcrossing. Although it is not possible to repeat experiments of this kind with equal results, it is nevertheless hoped that the investigations described here will reveal some new aspects of the subject.

II. MATERIALS AND METHODS

The stock used in the investigation was a wild type originally obtained from Algeria, but kept in the laboratory for several years under ordinary breeding conditions. From this stock 5 lines (series I, lines Nos. 1—5) were made up from normal animals and these lines were propagated by brother-sister mating during the whole experiment. Normal flies were always used as parents.

Some generations later 5 other lines (series II, lines Nos. 6—10) were made up from the Algeria main stock. These were also propagated by brother-sister mating, but so far as there were abnormal animals present, these were used as parents, those which seemed to deviate most from normal appearance were preferred.

At the same time two series with less intense inbreeding were started: series III, consisting of three lines, S1, S2, S3, where 5 female and 5 male deviant animals were taken as parents in each generation; and series IV, including the two lines S4 and S5, propagated by normal parents, 5 ♀♀+5 ♂♂. Line S5 was started 6 generations later than the others.

During the entire experiment the flies were reared in bottles with standard cornmeal-molasses-agar medium at 25° C. In the brother-sister matings the parents were allowed a mating period in vials at room temperature for 3—4 days before being transferred to bottles.

In order to record deviants, the flies were scrutinized at a magnification of 60×. In each generation about 50 of the first emerging flies of each sex were examined in each line. Sometimes, when fertility was low, this number was not available; sometimes, in series II and III, more flies were examined in order to secure the deviants needed as parents for the next generation. The frequencies of deviants are given as percentages, and it has not been judged necessary to weight these

TABLE 1. *Distribution of deviants on different organs in early generations of series I.*

The percentage distribution exceeds 100, since some animals deviate in more than one way.

Series	Generation	Total material			Distribution of deviants									
		Normal + dev.	Deviants		Bristles		Legs		Wings		Eyes		Miscellaneous	
			Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
I	4	551	241	43.7	214	88.8	48	19.9	26	10.8	25	10.4	12	5.0
	5	1033	96	9.3	79	82.3	8	8.3	9	9.4	0	0	4	4.2

TABLE 2. *Distribution of deviants on different organs in late generations of series I and II.*

The miscellaneous deviants in series II are almost all found in line 9. See text.

Series	Generation	Total material			Distribution of deviants									
		Normal + dev.	Deviants		Bristles		Legs		Wings		Eyes		Miscellaneous	
			Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
I	64	1044	209	18.3	201	96.2	6	2.9	0	0	2	1.0	4	1.9
	65	1001	166	16.6	157	94.6	8	4.8	2	1.2	0	0	0	0
II	54	1100	293	26.6	286	97.6	4	1.4	2	0.7	0	0	18	6.1
	55	1123	205	18.3	187	91.2	8	3.9	1	0.5	1	0.5	35	17.1

according to the number of animals recorded. Since the percentage values fluctuated within wide limits, the angular transformation has been applied to the raw percentages in all the calculations.

III. THE NATURE OF THE DEVIANTS FROM NORMAL APPEARANCE

In old cultures of the Algeria stock, flies with abnormal legs, wings and facets were found. In one old culture 102 animals were examined and 7 showed such abnormalities, i.e. 6.9 %. The offspring of these abnormal flies were quite normal, and it seems as if the manifestation of these abnormalities was due to the special conditions prevailing in the old stock culture. During the course of the experiment it was found that these gross malformations, although occasionally repeated, were very rare, and that the most common kind of deviations concerned the bristle formation. When all kinds of deviants were recorded, six different examinations from the main Algeria stock gave a mean frequency in per cent of 12.27 ± 2.58 deviant animals. The internal organs could not be included in the examination, nor could the details of the sex-combs and the genital apparatus, since this would have required a greater magnification, and this cannot be used on living flies without an undue increase of the time needed for the examination of each fly.

The distribution of abnormalities of different organs is shown in Tables 1—2. Among the early generations of series I, generation 4 had a high overall frequency of deviants, 43.7 %, whereas generation 5 had a low frequency, 9.3 %. The distribution was rather uniform throughout the experiment, and the generations shown can be looked upon as representative counts. Special abnormalities were only encountered in line 9 (see Table 2), which attained a high frequency of flies showing melanotic protuberant spots at the anterior border of scutellum, accompanied by malformed humeral bristles.

Deviations of the bristle pattern were for the main part extra bristles, seldom malformed bristles. Absence of bristles was very rare, if not due to a secondary loss of the bristle, in which case the socket on the body surface was still to be seen. Such cases were not included in the counts. The affected bristle groups were, in order of decreasing frequency: dorsocentrals, post-alars, verticals, orbitals, scutellars, post-verticals, supra-alars, humerals, pre-suturals, notopleurals. The dorsocentral cases amounted to about 50 % of the total.

The leg abnormalities consisted of thickened and compressed seg-

ments in one or several legs. Crooked legs were also found, when the femur or the tarsus was usually affected. The abnormal wings showed blisters or had failed to fold out. Sometimes it was difficult to distinguish developmental changes from secondary injuries. Doubtful cases were not recorded as deviants. All the eye abnormalities consisted of an irregular facet pattern, often only extending over parts of the eye. Such irregularities often occurred together with severe malformations of legs and wings. The miscellaneous deviations consisted of melanotic spots or unilateral malformations of tergites and sternites.

A sexual dimorphism was found to exist for the frequency of deviants. The females had a higher frequency of deviants, throughout the entire experiment. The difference is illustrated by a representative count from the same generations of series I and II, that are recorded in Table 2. In a total of 2231 females, 607 showed abnormalities, and in 2089 males, there were 245 deviants. The percentages are 27.2 for females and 11.7 for males, and the heterogeneity $\chi^2 = 162.3$, which for 1 degree of freedom corresponds to a probability much less than 0.001. Within each culture the male and the female frequencies were correlated (after angular transformation $r = 0.5929$, 54 d.f.). In the following analysis of the material, the sexes have been taken together to give an overall percentage. Since the recorded numbers of both sexes were about equal, the dimorphism cannot be supposed to bias the results.

IV. ENVIRONMENTAL INFLUENCES ON THE FREQUENCY OF DEVIANTS

It soon became apparent that the frequency of deviants fluctuated very much between generations, and that the fluctuations were similar, but not quite parallel for simultaneous cultures (Figs. 1—3). This is a well known phenomenon for many traits of low heritability in *Drosophila*. SOKAL and HUNTER (1958) have discussed a similar case, and have attributed the fluctuations to variations in the micro-environment afforded by the substrate, and the non-parallellism to a gene-environment interaction.

A comparison between corn-molasses and yeast medium was made in generations 10 and 11 of series I. The results reveal that the frequency of deviants is influenced by the nature of the substrate. When the larvae were cultured on a yeast suspension soaked up in tissue paper, the frequency of deviants was higher than for parallel cultures on corn-molasses medium (Table 3). The nature of the deviations was however

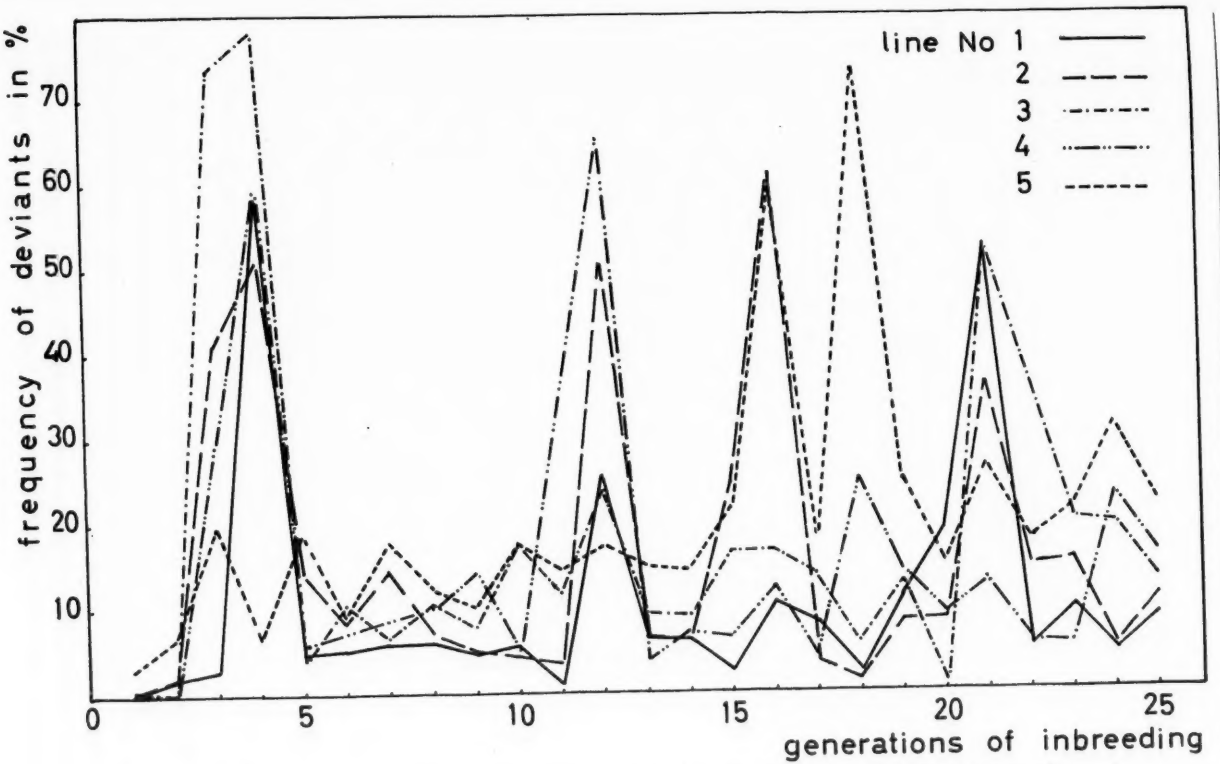

Fig. 1. Frequencies of deviants in the first 25 generations of series I; brother-sister mating with normal parents.

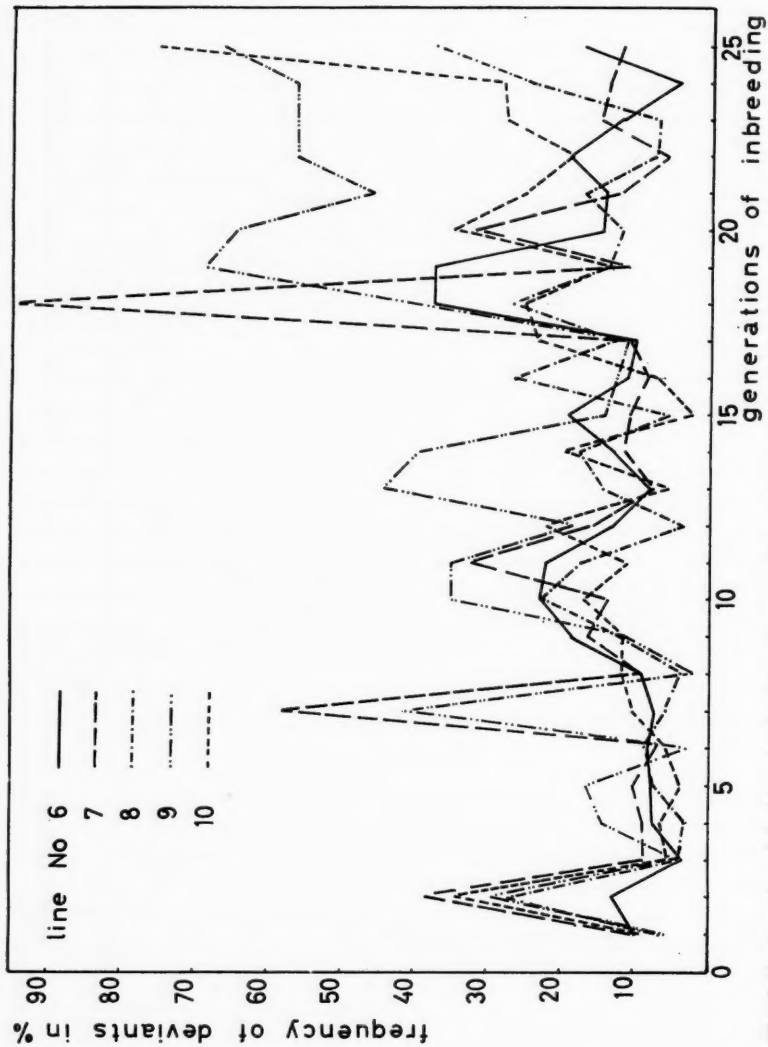

Fig. 2. Frequencies of deviants in the first 25 generations of series II; brother-sister mating with deviant parents.

the same on both media, viz. mostly extra bristles among the dorsocentrals, verticals and post-alars.

Since the yeast medium affords better conditions for larval development than the corn-molasses medium, the difference which was found

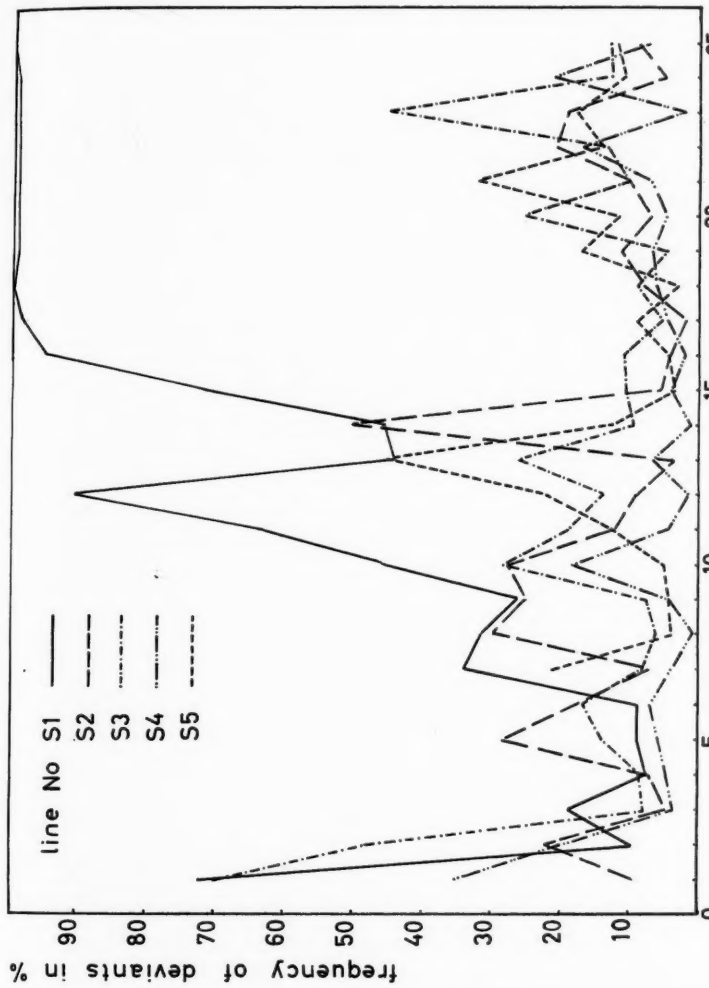

Fig. 3. Frequencies of deviants in the first 25 generations of series III (S1—S3) and series IV (S4 and S5). Series III has 5+5 deviant parents, series IV 5+5 normal parents in each generation.

in frequency of deviants might depend, entirely or partly, on the amount of food available during development. Another experiment was set up to test if the larval density could be an important factor for the occurrence of deviants. 4 ml of Kalmus medium (KALMUS, 1943) in vials was used as substrate for respectively 10, 20, and 40 newly hatched larvae from generation 16 of series I and generation 6 of series II. The imagos

TABLE 3. *Frequency of deviants in parallel cultures on two different media.*

Generation	Yeast			Corn-molasses			χ^2_{het}
	Examined	Deviants	% dev.	Examined	Deviants	% dev.	
10	1390	280	20.14	829	81	9.77	108.86**
11	791	170	21.49	469	43	9.17	74.42**

** $P < 0.01$

were counted and examined for deviations. Table 4 summarizes the results. The total percentages of deviants indicate a small reduction in frequency at higher densities and show a significant heterogeneity. This reduction has no parallel in the viability, which does not differ significantly between densities.

The frequency differences at varying larval densities are smaller than those found on different media, and therefore both the larval substrate and the larval density can be supposed to control the occurrence of deviants. However, it is probable that the microbial flora and the general consistency of the substrate are of greater importance, since a uniform medium was used and variations in larval density cannot account for all the fluctuations found in the material.

V. THE EFFECTS OF INBREEDING WITH AND WITHOUT SELECTION FOR DEVIANTS

The number of deviants was recorded during the first 39 generations of series I and during the first 30 generations of series II. Since the fluctuations in the frequency of deviants could not be controlled by ordinary uniformity of temperature and substrate, statistical means

TABLE 4. *Viability and frequency of deviants at different larval densities on 4 ml of Kalmus medium.*

	Number of larvae per vial			χ^2_{het}
	10	20	40	
Viability in %	83.1	80.3	81.5	1.39
Number of imagos	325	388	435	
Deviants in %	17.5	14.7	10.3	8.40*

* $P < 0.05$

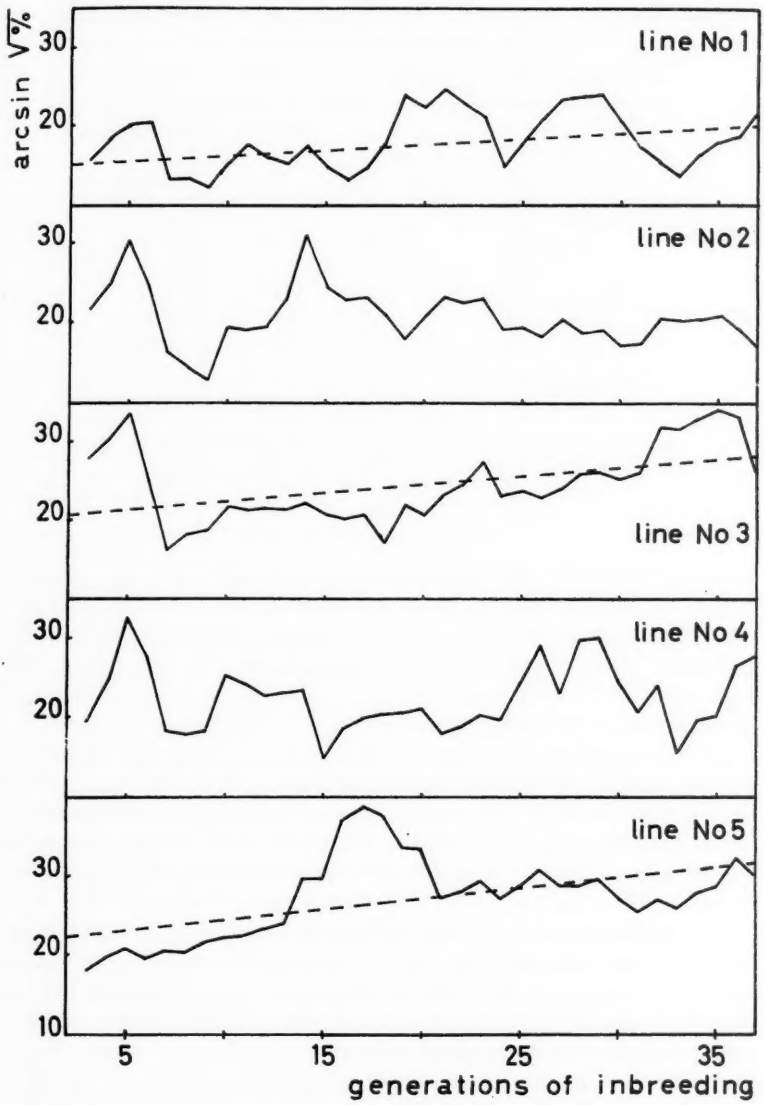

Fig. 4. Moving averages of transformed percentage values including 5 generations for the lines of series I. Where a significant regression on generation number is present, the regression line is indicated.

TABLE 5. *Regression coefficients and estimated frequencies of deviants in generations 0 and 25.*

Transformed values (arc sin $\sqrt{\%}$)

Inbreeding line	Regression coefficient	Estimated values of	
		y_0	y_{25}
Series I			
Line No 1	0.1174	16.08	19.02
„ „ 2	—	20.66	20.66
„ „ 3	0.2164	20.78	26.19
„ „ 4	—	22.40	22.40
„ „ 5	0.2619	22.56	29.11
Series II			
Line No 6	0.3662	17.76	26.92
„ „ 7	—	24.32	24.32
„ „ 8	0.2402	17.88	23.89
„ „ 9	1.2079	19.15	49.35
„ „ 10	0.7637	13.99	33.08

were used to remove the fluctuations from the data when the influence of inbreeding and selection was analysed. In Figs. 4—6 the raw data from figures 1—3 have been smoothed by computing moving averages, including 5 generations. It then became possible to get a more clear impression of the trends and to compute the regression of the frequency of deviants on generation number. In the cases where a significant regression was obtained, the regression lines are indicated on the diagrams. Table 5 shows the regression coefficients and the estimated frequencies (y-values) in generation 0 and 25. Since the number of generations is different in series I and series II the mean frequencies cannot be directly compared. Table 6 gives the joint analysis of variance and co-variance.

It is clear from the diagrams and the analysis that inbreeding results in an increase in the frequency of deviants, both in series I and series II. In series I, where normal animals have been used as parents, this increase cannot be due to any kind of selection, and must therefore be a manifestation of the increasing homozygocity. The difference between regression coefficients is significant within series I, and in two of the lines, No. 2 and No. 4, there is no regression on generation number. This implies a genotype-inbreeding interaction, so that different genotypes are differently influenced by the inbreeding.

TABLE 6. *Joint analysis of variance and co-variance for series I and II.*

Cause of variation	Degrees of freedom	Mean square	Variance ratio
Joint regression	1	1246.57	76.95***
Difference between regression coeff.	9	280.52	17.32***
Between series I and II	1	818.30	3.84
Within series	8	213.29	
Within series I	4	73.61	4.54**
Within series II	4	352.97	21.79***
Difference between means	9	636.78	39.31***
Between series I and II	1	644.11	1.01
Within series	8	635.86	
Within series I	4	432.38	26.69***
Within series II	4	839.34	51.81***
Error	285	16.20	

** $P < 0.01$; *** $P < 0.001$

The significant differences in mean frequency indicate a genetic heterogeneity between lines, and thus a genetic determination of the liability to developmental accidents, or of the intensity of the "developmental noise".

The regression coefficients and the mean frequencies of series II are very much the same as in series I, and no significant differences are to be found between series. Also in this series one of the lines, No. 7, shows no regression on generation number. In line No. 10 the increase is very marked, but the deviants were not found to be of a specific kind. Only in line No. 9 the selection for deviants seems to have been effective in fixing a fairly penetrant gene constellation causing a specific abnormality, viz. the melanotic spots at scutellum and the malformed humeral bristles mentioned on page 515. The occurrence of this character does however not exceed 80 % in females and 50 % in males. It is evident that natural selection for viability acts against the expression of the trait, for after a maximum frequency around generation 20 accompanied by low fertility (so low that the inbreeding had to be relaxed for some generations) the frequency of deviants is reduced (Fig. 5). The computed regression on generation number is therefore not valid for later generations of this line.

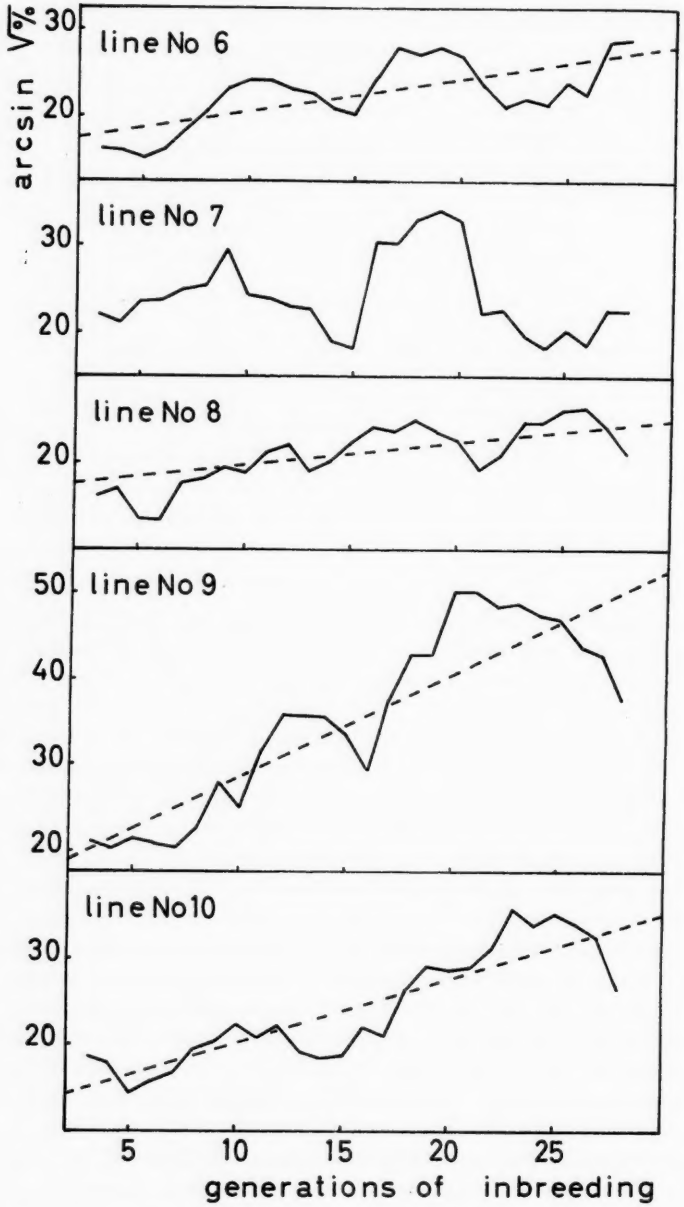

Fig. 5. Moving averages of transformed percentage values including 5 generations for the lines of series II. Significant regression lines are indicated.

The deviants selected for in series II, with the exception of those in line No. 9 and possibly No. 10, are not caused by the action of a main gene, but their occurrence is rather a manifestation of developmental instability. This instability is not much influenced by the selection, for the mean levels do not differ in series I and II. It seems to be determined by the genotypical balance and by the degree of inbreeding. The lines with no regression on generation number, i.e. those which show no influence of inbreeding, usually start at a higher frequency level (Table 5).

VI. THE EFFECTS OF SELECTION

In series III and IV the frequency of deviants was recorded during 37 generations (for line S5 six generations less). The foundation population has been broader, 5 ♀♀+5 ♂♂, and the degree of inbreeding less than in the two previous series. The theoretical disintegration rate of genetic variance is about 5 % per generation. When deviants are used as parents, this breeding system should provide greater possibilities to secure main genes causing malformations. This has succeeded in line S1, where a completely penetrant gene for extra bristles on scutellum and thorax became fixed. A genetic analysis showed this gene to be situated in chromosome III between *pink* and *spineless*. The gene lowers the frequency of crossing over in this interval and in the adjacent interval *spineless—ebony*.

In the other two lines of series III no main gene for abnormalities was secured. Smoothed diagrams of these lines are shown in Fig. 6; their regression coefficients on generation number and estimated frequency values in generations 0 and 25 appear in Table 7. In line S2 the regression on generation number is not significant, nor is it in the two lines of series IV. A joint analysis of variance and co-variance for these four lines was made and is shown in Table 8. The joint regression is significant although only line S3 has a definite regression coefficient. The difference in mean levels can be attributed to line S4, which has a very low frequency throughout.

Selection on the broader basis thus has not been generally effective in securing main genes for malformations. They must be supposed to be rare in the original population. Where no specific abnormality was fixed, the selection lines of series III did not show a higher frequency of deviants than those of series I and II. Selection thus was not more effective in raising the frequency of deviants than inbreeding. Selection for normality, which can be regarded as a control, and which is repre-

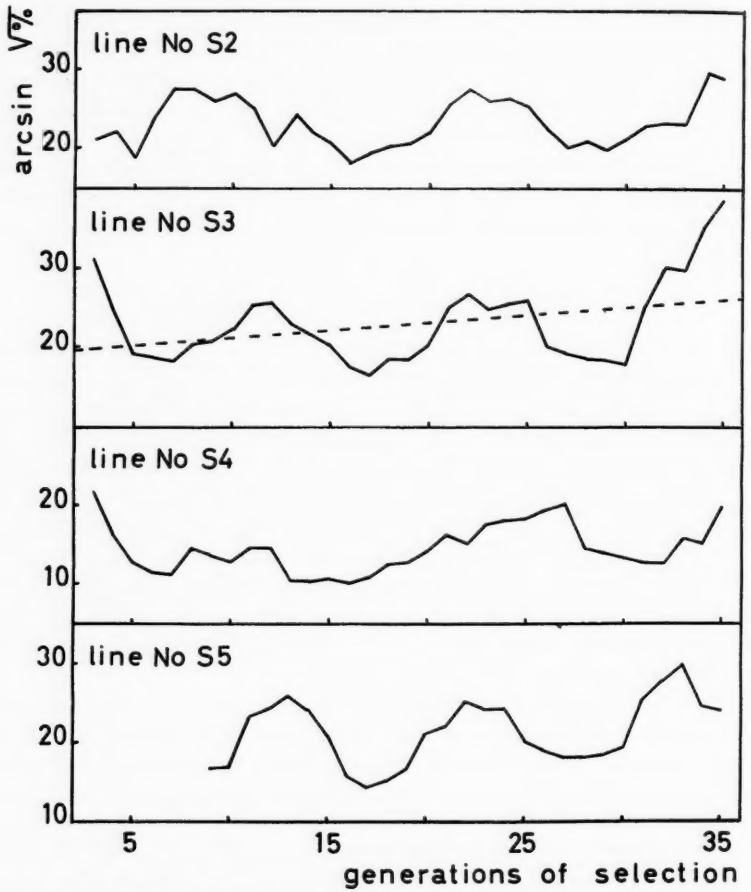

Fig. 6. Moving averages of transformed percentage values including 5 generations for lines S2—S5. Regression line indicated for line S3.

sented by series IV, maintained the frequency of deviants at a rather low level, but only that of line S4 is definitely lower than the others.

VII. DIFFERENT CRITERIA OF DEVELOPMENTAL STABILITY

The analysis of the frequency of unspecific morphological deviants given in the previous sections, has shown that the occurrence of deviants

TABLE 7. *Regression coefficients and estimated frequencies of deviants in generation 0 and 25.*

Transformed values (arc sin $\sqrt{\%}$)

Selection line	Regression coefficient	Estimated value of	
		y_0	y_{25}
Series III			
Line No S2	—	23.29	23.29
„ „ S3	0.2013	19.80	24.83
Series IV			
Line No S4	—	14.57	14.57
„ „ S5	—	20.63	20.63

is little influenced by selection, but that their frequency may be increased by close inbreeding. The results suggest that this frequency is a criterion of developmental stability comparable to asymmetry for bilateral structures and non-genetic variation. The inbred Algeria lines from series I and II were therefore used for an investigation of the agreement between different criteria of developmental stability in inbred and crossbred genotypes. In the comparison were included: 1. frequency of morphological deviants; 2. asymmetry in number of sternopleural bristles on right and left side of the body; 3. variation in the total num-

TABLE 8. *Joint analysis of variance and co-variance for series III and IV.*

Line No S1 not included

Cause of variation	Degrees of freedom	Mean square	Variance ratio
Joint regression	1	149.60	10.06**
Difference between regression coeff.	3	17.31	1.16
Difference between means	3	560.74	37.70***
Between series III and IV	1	994.52	2.89
Within series	2	343.85	
Within series III	1	0.07	—
Within series IV	1	687.62	46.23***
Error	118	14.87	

** $P < 0.01$; *** $P < 0.001$

ber of sternopleural bristles within genotypes, which may be regarded as a non-genetic component of variation; 4. sensitivity to the organophosphorous insecticide 0,0-diethyl 0-*p*-nitrophenyl phosphate (paraoxon). Resistance to a poison can be regarded as a criterion of the buffering capacity of the genotype, and therefore is of interest in this connection.

When the experiments were performed the lines of series I had been inbred for 45 to 50 generations, those of series II for 35 to 40 generations. In order to include in the investigation outcrosses to a non-related strain, the inbred line Ör was introduced. This line, which originated from the Swedish wild type stock Örebro, had been made isogeneous by outcrosses to marked inversions, and had subsequently been propagated by brother-sister mating for more than 20 generations.

1. Frequency of deviants, asymmetry and non-genetic variation

The study of frequency of deviants, asymmetry and non-genetic variation included in all 83 cultures from 52 different genotypes. Sternopleural bristles were counted on 20 females from each culture, the right and the left side number being recorded separately. From the same cultures the percentage frequency of deviants was recorded from examination of 50 females. The frequency of deviants in this study is thus not directly comparable to that of the preceding sections which included males as well as females.

In each culture the sums of squares for the differences between right and left side (SS_D) and for the total numbers (SS_T), as well as the mean difference between right and left side disregarding sign (A), were computed from the sternopleural bristle numbers.

Asymmetry of sternopleural bristle numbers has earlier been studied by MATHER (1953) and THODAY (1955). MATHER used SS_D (by him designed V) as a measurement and he found that it showed no definite regression on total bristle number. However, the maximum difference in mean total bristle number in his lines only amounted to a little over one bristle. THODAY used A and, in cases where the mean total bristle numbers differed widely, A/T (T=mean total bristle number). Using the present material, SS_D and A for each separate culture were plotted against each other. The relationship was slightly curvilinear, but within the existing range the two measurements could be considered as equivalent.

The question of correcting for differences in total bristle number is of great importance. The lowest bristle number was found in the Ör line

TABLE 9. *Developmental stability of inbred and crossbred flies.*

Breeding type	Number of different genotypes	Criterion of stabitity					
		Morpho-logical de-viants in %	Asymmetry measured as			Non-genetic variation measured as	
			SS_D	A	A/T ×100	SS_T	CV
Inbred Algeria lines, I_A	10	15.0	42.9	1.13	5.50	59.6	36.4
Inbred Ör line, $I_{Ö}$	1	13.0	19.2	0.72	6.48	30.3	49.6
Crosses between Algeria lines, C_A	32	10.4	44.4	1.15	5.67	59.3	37.3
Outcrosses to Ör line, $C_{Ö}$	9	1.1	15.6	0.64	4.18	23.2	31.2

which had about 11 bristles. The crosses between Ör and the Algeria lines had means amounting to 14—16 bristles, and the Algeria lines themselves, as well as crosses within them, had means ranging from 18 to 24 bristles. There is thus a great variation present in the material, and a reliable correction is necessary for a sound comparison over the whole material. This is true both for the asymmetry measurements, SS_D and A, and for the non-genetic variation, SS_T, which is known to be influenced by the mean (RASMUSON, 1952).

The corrections applied to the present material were, for the non-genetic variation CV, the coefficient of variation, computed as the standard deviation in percentage of the mean, and for the asymmetry A/T, the correction used by THODAY. However, none of these corrections is quite accurate; both tend to overestimate the influence of the mean. This is revealed by the inbred line Ör, which, having the lowest mean, reaches the highest values both of CV and A/T. The corrections may however be considered adequate for the present, since they do not modify the most important conclusions which can be drawn from the data.

A number of deductions may be made from the comparisons set out in Table 10. The inbred Algeria lines and the Ör line do not differ except in CV, which obviously is a biased estimate of non-genetic variation in $I_{Ö}$, because of its low mean. The comparison I_A—C_A reveals that the crossbreeding between the Algeria lines has little effect on asymmetry and non-genetic variation, but lowers the frequency of morphological deviants. This is to be expected, since the lines of series II have been selected for deviants, and in some cases at least they seem to have

TABLE 10. *Differences between breeding types for frequency of morphological deviants (% D), asymmetry (100 A/T), and non-genetic variation (CV).*

Comparison	Degrees of freedom	% D		100 A/T		CV	
		Diff.	t-value	Diff.	t-value	Diff.	t-value
$I_{Ö}$—I_A	31	−2.0	0.526	0.98	1.906	13.2	9.093***
I_A—C_A	60	4.6	2.361*	−1.7	0.624	−0.9	0.621
C_A—$C_{Ö}$	48	9.3	12.245***	1.49	3.990***	6.1	4.026***

* $P<0.05$; *** $P<0.001$

accumulated gene combinations which increase the frequency. These combinations are not transmitted to crossbred offspring, and therefore apparently have been based on homozygocity. Finally the comparison C_A—$C_{Ö}$ gives highly significant differences for all three kinds of criteria. The outcrossing to an unrelated genotype has increased the stability of development much more than the crossbreeding among the Algeria lines. Since the $C_{Ö}$ group has a low mean bristle number, the comparison cannot be biased by the applied corrections.

2. Sensitivity to para-oxon

This part of the investigation has been carried out by Mrs. M. SVENSSON who kindly has permitted me to cite her results. The sensitivity of different genotypes was tested during the larval stage by rearing batches of 50 newly hatched larvae on media containing para-oxon in concentrations varying from 0.1 to 0.5 parts per million (ppm). The toxicity

TABLE 11. *Mean lethal doses (LD_{50}) of para-oxon in ppm.*

Genotype I (I_ANo or $I_{Ö}$)	LD_{50}		Genotype C_A ($I_A \times I_A$)	LD_{50} Exp. 1	Genotype $C_{Ö}$ ($I_A \times I_{Ö}$)	LD_{50} Exp. 2
	Exp. 1	Exp. 2				
1	0.235	0.316	1× 2	0.310	1×$I_{Ö}$	0.263
2	0.300	0.355	1× 9	0.363	2×$I_{Ö}$	0.363
9	0.190	0.209	1×10	0.296	9×$I_{Ö}$	0.355
10	0.340	0.339	2× 9	0.289	10×$I_{Ö}$	0.437
$I_{Ö}$	—	0.263	2×10	0.363	—	—
—	—	—	9×10	0.363	—	—
Mean	0.283			0.331		0.355

I_A=inbred Algeria line; $I_{Ö}$=inbred Örebro line

was computed from the difference between the original number of larvae and the observed number of imagos in each batch. The mean lethal dose (LD_{50}) was estimated for each genotype according to the maximum likelihood method (FINNEY, 1952). Two separate experiments were performed, the first one included lines Nos. 1, 2, 9, 10, and crosses between these: the second one included, in addition to the four Algeria lines, the line Ör and crosses between the Ör and the Algeria lines. The estimated values of LD_{50} from these two experiments are shown in Table 11. The inbred lines have the lowest values of LD_{50}, i.e. they are the most sensitive to the poison. Among the crossbred genotypes the outcrosses to the Ör line, group $C_{Ö}$, are a little less sensitive than the C_A group. An analysis of variance, performed on the LD_{50} values, revealed a significant difference between inbred and crossbred genotypes ($P<0.05$) but no significance for the difference between C_A and $C_{Ö}$.

VIII. DISCUSSION

Numerous investigations have shown that, when fitness or selective value is concerned, heterozygotes usually are superior to homozygotes, even if the genomes united in the heterozygote originate from geographically different populations (for examples, see DOBZHANSKY, 1955). No such generality seems to exist for morphological variations. REEVE and ROBERTSON (1954) found the non-genetic component of variation to be reduced in the F_1 generation for wing length but not for abdominal sternite bristles. LEWONTIN (1956), also studying sternite bristles, found in the F_1 generation a higher non-genetic variance between individuals, but a lower variance between segments of individual flies, than in the homozygous combinations. MATHER (1953) found a reduction in asymmetry for sternopleural bristles in the F_1 between his original strains, while THODAY (1955), using the same character, obtained varying results, "not explicable in terms of degrees of homozygocity".

The different aspects of morphological variation which have been studied in this investigation, frequency of minor deviants, asymmetry of sternopleural bristles, and within-culture variation of total sternopleural bristle number, have no immediate significance for the fitness of the individual. Sensitivity to poison on the other hand, as reflecting the buffering capacity of the individual, is more closely related to fitness.

The results of the present investigation on the frequency of unspecific deviants indicate that this is influenced by the genotypical balance and

by the degree of inbreeding, and that there is a marked genotype-inbreeding interaction. Selection has little effect, except in accumulating rare gene-combinations causing specific deviations. These facts place the unspecific deviants in the category of developmental noise, where the asymmetry of sternopleural bristles and the non-genetic variation in bristle number may also be included.

The outcrosses between the different inbred Algeria lines and between these and the unrelated Ör line now offers a possibility to compare these different aspects of developmental instability with sensitivity to a poison, which reflects the buffering capacity against environmental challenges and is an aspect of canalization or homeostasis. The following comparisons may be set up:

frequency of morphological deviants	$I > C_A > C_{\ddot{o}}$
asymmetry	$I = C_A > C_{\ddot{o}}$
non-genetic variation	$I = C_A > C_{\ddot{o}}$
sensitivity to poison	$I > C_A \geqslant C_{\ddot{o}}$

The difference between I and C_A for frequency of deviants can, as was stated earlier, be accounted for by the previous history of selection. Disregarding this discrepancy, the first three characters react in the same way towards outcrossing, whereas the sensitivity to poison differs in showing the greatest discrepancy between I and C.

Since the inbred lines have been kept for many generations without showing any severe reduction in viability, it must be supposed that they have overcome the dangers of inbreeding by accumulation of vital homozygous combinations. However, the varying frequencies of deviants as well as the mean bristle numbers indicate a genetic variability between the lines, even between those of common origin. It is therefore obvious that the degree of heterozygocity must have increased in the crossbred group C_A. This increase has had no influence on the morphological variation, but causes a significant reduction in sensitivity to poison. On the other hand, in the outcrosses to an unrelated genotype, group $C_{\ddot{o}}$, the morphological variation is reduced in all its three aspects, whereas the sensitivity to poison is little reduced in comparison to the C_A group.

The conclusion to be drawn seems to be that the buffering capacity is more directly related to the degree of heterozygocity than are the different aspects of morphological instability. Possibly this can be related to their importance for fitness, which must be greater for buffering capacity than for minor morphological variations. Whereas the

buffering capacity is always of selective value, minor deviations in morphology may not have been selected against within the Algeria population, and therefore such characters are not heterotic in the crosses between the lines from this population. In combination with the quite unrelated genome from the Ör line other sources of gene co-operation may have become available, which makes the developmental process more rigid. Here it must be noted that both the Algeria and the Örebro strains through a long period of laboratory breeding have been adapted to the same environment. Therefore the upset of the genetic co-operation which is sometimes found when strains adapted to different environments are crossed, is not to be expected.

SUMMARY

The frequency of unspecific morphological deviants has been studied in different lines descended from the wild type stock Algeria of *Drosophila melanogaster*.

The morphological anomalies mostly concerned the bristle formation, more seldom legs, wings and eyes. The nature of the breeding media as well as the larval density were found to influence the frequency of deviants, but could not entirely account for the great fluctuations found between generations. For an analysis of the effects of inbreeding and selection the data were smoothed by computing moving averages including 5 generations. These were used for an analysis of regression on generation number.

During a sequence of 30—40 generations brother-sister mating was found to increase the frequency of deviants. The use of deviant parents had little effect, except in one case where a specific abnormality appeared. Selection on a broader basis, 5 females and 5 males, in one case led to fixation of a gene causing extra bristles. In two other lines the frequency of deviants was little changed by selection. Breeding by 5+5 normal parents led to the maintainance of a low frequency of deviants.

It was concluded that the frequency of deviants could be used as a criterion of developmental stability. In a special experiment it was compared to two other criteria of morphological stability, viz. bilateral asymmetry and non-genetic variance of sternopleural bristle number, and one criterion of buffering capacity, viz. sensitivity to the organophosphorous insecticide para-oxon. In this experiment three different breeding types were included: the inbred lines from the Algeria stock and one inbred line from the unrelated stock Örebro (group I); the

crossbred offspring from crosses between different Algeria lines (group C_A); the crossbred offspring from crosses between the Algeria lines and the Örebro line (group $C_{Ö}$).

The three criteria of morphological stability reacted in the same way to outcrossing, viz. there was no difference between groups I and C_A, but the outcrossing to the unrelated line increased the stability of group $C_{Ö}$. The buffering capacity, on the other hand, was increased in both C_A and $C_{Ö}$. It is inferred that its greater importance for fitness has made the buffering capacity more heterotic than the morphological stability.

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OCCURRENCE OF BINUCLEATE AND MULTINUCLEATE POLLEN MOTHER CELLS IN HORDEUM

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INTRODUCTION

DURING the course of a cytogenetical investigation involving normal and a number of semi-sterile and completely sterile barley mutants a few pollen mother cells (PMC) were always found to be bigger in size than normal and contained two or more chromosome complements.

A cytological investigation of the PMC in this experimental material was undertaken to determine the frequency with which this phenomenon occurred.

The mutants were induced by various doses of X-radiation and neutron exposure in erectoides-14 and erectoides-23. Sterility in the mutants was due to heterozygosity for one or two reciprocal translocations, or to asynapsis, desynapsis and trisomic condition. In addition some of the mutants showed normal meiosis but may have possessed one or more genes causing pollen degeneration.

Barley spikes going through meiosis were fixed in acetic alcohol (3: 1) solution, stained in leuco-basic-fuchsin after acid hydrolysis and the anthers squashed in 45 % acetic acid. Only those PMC showing binucleate or multinucleate condition and with intact cell wall were recorded in this study to reduce errors due to squashing.

CYTOLOGICAL OBSERVATIONS

A study of meiosis revealed that the great majority of PMC were uninucleate and showed the chromosome complement and meiotic behaviour normal for barley. A small proportion of PMC had two or more nuclei.

TABLE 1. *Frequency of binucleate and multinucleate pollen mother cells.*

	Number of PMC observed	Frequency (%)
Uninucleate	1631	97.3
Binucleate	41	2.4
Trinucleate	2	0.1
Quadrinucleate	2	0.1
Total	1676	

Frequency of PMC containing one, two or more nuclei is shown in Table 1. Most of the pollen mother cells are uninucleate (97.3 %). A few are binucleate (2.4 %, Fig. 1 a—c) or multinucleate (0.2 %, Fig. 1 d).

Binucleate and multinucleate PMC occur with about equal frequencies in both normal and mutant barley. Therefore, the data presented in Table 1 have been combined from the two materials.

The nuclei may be synchronised in meiotic division cycle or they may be non-synchronised. No differences were observed in this respect between normal and mutant barley. In Fig. 1 a, a PMC can be seen containing two nuclei with their divisions synchronised, the chromosomes being at pachytene. They may be non-synchronised as illustrated in Fig. 1 c. The cell illustrated in Fig. 1 c was found in a trisomic plant, one nucleus being in late Metaphase I or early Anaphase I and the other at mid Anaphase I. Fig. 1 d illustrates a trinucleate cell found in normal barley; two of the three nuclei (left of the photomicrograph) having a synchronised division are at Anaphase I and the third (right of the photomicrograph) showing a non-synchronised division is at Metaphase I.

The plane of orientation of chromosome complements of the nuclei during the meiosis may be parallel to each other (Fig. 1 b) or at right angles to each other (Figs. 1 c and d). The spindle apparatus of the nuclei may be perfectly synchronised and lie so close to one another as to be indistinguishable from each other (Figs. 1 b and d, nuclei to the left), or it may be quite separate and non-synchronised (Fig. 1 d). The distance between the nuclei does not seem to determine their synchronisation or non-synchronisation within one PMC. Thus, the nuclei may be far apart and show a synchronised division cycle as shown in Fig. 1 a, or they may be far apart and be non-synchronised (Fig. 1 d). On the other hand, the nuclei may be close to one another and be synchronised as shown in Fig. 1 b and the two nuclei at left in Fig. 1 d, or

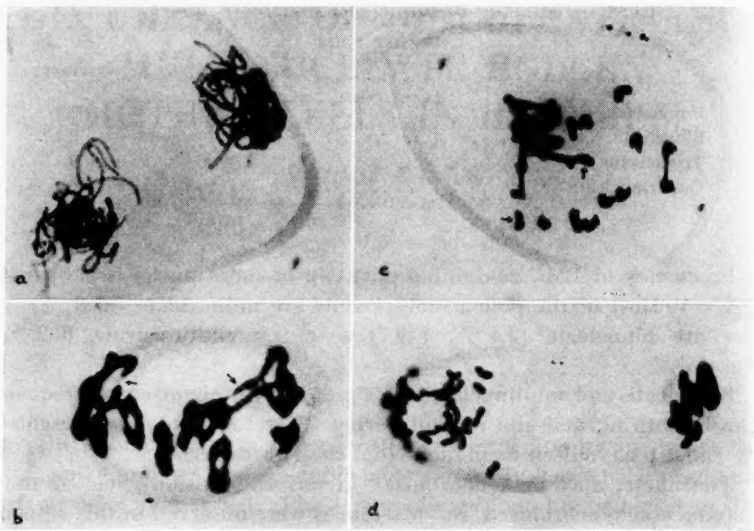

Fig. 1. a: Photomicrograph of a binucleate pollen mother cell in normal barley. The chromosome complements of the two nuclei are synchronised, chromosomes being at pachytene. — b: Photomicrograph of a binucleate pollen mother cell in a barley mutant regularly having one ring of six at meiosis (two rings of six in the binucleate cell are indicated by the darts). The chromosome complements of the two nuclei are synchronised, chromosomes being at Metaphase I. — c: Photomicrograph of a binucleate pollen mother cell in a trisomic barley mutant. The extra chromosome in the two nuclei is indicated with darts. The chromosome complements of the nuclei are slightly non-synchronised, one being at late Metaphase I or early Anaphase I and the other at mid Anaphase I. — d: Photomicrograph of a trinucleate pollen mother cell in normal barley. The chromosome complements of two of the nuclei (left) are synchronised at Anaphase I and are included on a common spindle, whereas those of the third nucleus (right) are non-synchronised with the other two, the chromosomes being at Metaphase I.

they may be situated close to one another and still be non-synchronised (Fig. 1 c).

DISCUSSION

Binucleate and multinucleate pollen mother cells have not been reported in barley before. However, they have been noted by many investigators in different materials. These reports are listed in Table 2.

The frequency of binucleate and multinucleate PMC have not been reported before although their occurrence has been confirmed several

TABLE 2. *Instances of binucleate pollen mother cells.*

Experimental material	*Authors*
Oenothera lamarckiana	GEERTS, 1909
Lactuca	GATES and REES, 1921
Celsia	HÅKANSSON, 1926
Verbascum	HÅKANSSON, 1926
Zea mays	RANDOLPH and MCCLINTOCK, 1926
Oenothera lamarckiana	HÅKANSSON, 1927
Raphanus×*Brassica*	KARPECHENKO, 1927
Nicotiana tabacum (haploid)	RUTTLE, 1928
Iris Kaempferi	INARYAMA, 1929
Triticum×*Aegilops* (5x)	KAGAWA, 1929
Zea mays (asynaptic)	BEADLE, 1930
Prunus avium	DARLINGTON, 1930
Oenothera franciscana	DAVIS and KULKARNI, 1930
Crepis capillaris (haploid)	HOLLINGSHEAD, 1930
Avena sativa (6x—2)	NISHIYAMA, 1931
Chrysanthemum ornatum	SHIMOTOMAI, 1931
Chrysanthemum japonense	SHIMOTOMAI, 1933
Triticum vulgare×*Secale cereale*	KATTERMANN, 1933
Triticum aegilopoides×*Aegilops squarrosa* (5x)	KIHARA and LILIENFELD, 1934
Cannabis sativa	MEDWEDEWA, 1934
Phacellanthus tubiflorus	MATSUURA, 1935
Oryza sativa	NANDI, 1937
Pyrus communis	CRANE and THOMAS, 1939
Lolium rigidum	JENKINS and THOMAS, 1939
Secale cereale	MÜNTZING and PRAKKEN, 1941
Triticum compactum×*Secale cereale*	NAKAJIMA, 1954
Allium ascalonicum	DARLINGTON and HAQUE, 1955
Secale cereale (inbred genotype)	REES, 1955
Avena barbata×*strigosa* ssp. hirtula	HOLDEN and MOTA, 1956
Triticum turgidum×*Agropyron intermedium*	ARMSTRONG and HATTERSLEY-SMITH, 1958
Antirrhinum majus (haploid)	RIEGER, 1958
Gossypium hirsutum×*thurberi*	SARVELLA, 1958
Capsicum annuum (virus infection)	SWAMINATHAN *et al.*, 1959
Hordeum vulgare (normal and mutants)	KAMRA (present investigation)

times (cf. Table 2). It has been, therefore, the general belief that this is a rare phenomenon. The present investigation indicates that binucleate PMC may occur with regularity in barley. About 2.4 % of all the PMC studied fall under this group. They were seen in almost all of the material studied.

It is difficult to say if gametes arising out of such cells are functional. In a very low frequency they may be expected to effect fertilization and thus give rise to aneuploid and polyploid progeny in a population. Binucleate and multinucleate cells may also be expected to occur in a low proportion of egg mother cells (EMC).

It has been noted in several of the investigations (MATSUURA, 1935; HOLDEN and MOTA, 1956; RIEGER, 1958) that one of the nuclei of a binucleate cell usually undergoes degeneration and the other goes on with the normal course of meiosis. This abnormality is believed to be due to a cytoplasmic gradient in a binucleate cell. Such an abnormality has not been noted in the present material.

These cells could originate in different ways. Virus infection is known to increase the occurrence of binucleate PMC in *Capsicum* (SWAMINATHAN *et al.*, 1959). Such an origin can be eliminated for binucleate PMC in the present study.

Cytomixis or transfer of chromatin from the nucleus of one PMC into the cytoplasm of an adjacent PMC can also give rise to the binucleate condition (KATTERMANN, 1933; KIHARA and LILIENFELD, 1934; MATSUURA, 1935; NANDI, 1937; SARVELLA, 1958). Although cytomixis has been observed in some mutants of barley, this explanation does not hold true in the present observations since no remnants of cell wall could be seen inside such a cell and the different nuclear membranes appear to be intact. Their origin can be attributed to the failure of cell wall formation at pre-meiotic mitosis, giving rise to binucleate cells with sister nuclei in a common cytoplasm.

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SUMMARY

Binucleate and multinucleate pollen mother cells (PMC) were found in a cytogenetical investigation of normal and mutant barley (erectoides-14 and erectoides-23). These cells were found to occur in all the types studied in a low frequency. No signs of degeneration were noted in any of the nuclei of a multinucleate PMC. The nuclei may be synchronised or non-synchronised in their meiotic division. The binucleate condition is attributed to the failure of cell wall formation at premeiotic mitosis.

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AN EVALUATION OF SOME ANTHROPOLOGICAL TRAITS USED IN PATERNITY TESTS

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I. INTRODUCTION

ANTHROPOLOGICAL traits were first used in legal medicine in 1926 by RECHE in Austria and in 1928 by v. VERSCHUER in Germany (cf. SCHADE, 1954). The methods were further elaborated by FISCHER, MOLLISON, v. VERSCHUER, KRAMP, LENZ and SCHWIDETZKY in Germany, and by WENIGER and GEYER in Austria. Historical details will be found in publications by SCHADE (1954) and HARASSER (1957). ESSEN-MÖLLER and QUENSEL (1939) developed a formula by which the probability of paternity could be calculated. This formula was primarily intended for simple Mendelian traits, *e.g.* blood groups.

In Sweden the use of anthropological paternity tests, in addition to blood groups, was legally recognized in 1950. Unfortunately, experience in the form of accumulated family data is lacking. ESSEN-MÖLLER (1941), however, has studied the genetics of some anthropological traits using the twin method. The present investigation is a study of the genetics of some traits generally used for medico-legal purposes. To facilitate a direct comparison with actual paternity cases, the data have been collected according to the pattern in which these appear.

The material consists of data from one hundred Swedish families with which contact was established through the Children's Health Center in Uppsala. The parents and one child have been examined in each family. The mean age of the fathers, mothers and children is shown in Table 1. The age distribution of boys and girls is given in Table 2. The mean age of the children was about 3 years, which is the age when anthropological paternity tests are usually carried out. The low age of the children in the present material is important for the study of age differences as a source of error in paternity tests. The parent sample was distributed over most Swedish counties (cf. Table 3)

with an overrepresentation from the areas around Stockholm and Uppsala. The sample might, however, be considered rather representative for the Swedish population.

II. METRICAL TRAITS

Seven metrical traits have been recorded. The instruments used for the measurements were the spreading caliper, the sliding compass and an angle-instrument. Linear measurements were made in millimetres, and angles were measured with an accuracy of five degrees.

The *cephalic index* is the most well-known trait of the classical physical anthropology. Our data show that this trait varies with sex and age, which is in accordance with most other data. The index was higher among the females than males, and there was a tendency towards dolichocephaly with increasing age.

The *nose-lip index* was based on two measurements, the distance between the *angulus medialis oculi* and the root of the *alae nasi*, respectively, to the upper border of the mucous membrane of the upper lip. A low index indicates a long upper lip and a relatively short nose. ROMANUS (1952 a) has studied this index in a population sample of Swedish students and in the Swedish royal family and claimed that a low index is dominant.

The *interocular-biorbital index* (cf. ROMANUS 1953) is a measure of the relative distance between the eyes. ROMANUS (1953) has discussed the value of the interocular-biorbital index in cases of disputed paternity and suggested that extreme values might be of diagnostic value.

The *index* $\frac{\textit{bi-acromial diameter} \times 100}{\textit{inter-iliocristal breadth}}$ measures the relative breadth of the shoulders.

The *ear index* is based on the measurements of the length and breadth of the ear. The higher the index, the shorter and broader is the ear.

The sex differences (differences between fathers and mothers) have been summarized in Table 4. There is a significant difference ($P \leqq 0.05$) for all traits except for hyper-extensibility of the distal thumb joint.

The age differences (differences between fathers—children and mothers—children) are shown in Tables 5 and 6. Significant differences were found between both fathers—children and mothers—children for five traits. For two traits, there was a significant difference only between mothers and children. It should be observed that the relative

breadth of the shoulders in the children was the same as in the men, whereas the women showed lower figures.

For the three bilateral traits, the differences between the left and the right side were calculated (Table 7) for all individuals. The ear angle showed no significant bilateral variation. The hyperextensibility of the distal thumb joint was more pronounced on the left side. The difference for fathers, however, was not significant. The ear index was higher on the left side, although the difference for the mothers was not significant.

The correlations between fathers and children are shown in Table 8. The correlations for total finger ridge count (BÖÖK, 1957) have been included in this table. All correlations were positive, and those between fathers and children are significant for all traits except the cephalic index. In most cases, however, the values are low as compared to the expected ones, indicating that environmental factors cause a substantial part of the variation. It is important to compare with the figures for total finger ridge counts, which are close to expectation. It seems likely that environmental factors influence the variation of finger ridges less than *e.g.* variations in the shape of the head.

For all traits, either sex, age or bilateral variations were of such a magnitude as to make elaborate mathematical corrections necessary should these traits be used in practical tests. Very large samples would be required as a basis for such calculations. Furthermore, it is likely that unknown, non-genetical factors interfere to a great extent. Thus the bilateral variations concerning the extensibility of the distal and proximal thumb joint probably depend upon the different degree of training of the right and left hand.

We feel that the use of metrical traits for medico-legal purposes would involve such practical difficulties that they cannot be recommended at present. We also doubt that the time-consuming and expensive collection of extensive and unbiased family data, necessary for working out an empirical procedure, would be a worthwhile undertaking, specifically with regard to the critical information of legal value that can be extracted. This does not, of course, include any criticism or evaluation of the basic research in this field.

III. QUALITATIVE TRAITS

Seventeen qualitative traits among those currently used in paternity tests were selected and examined.

1. *Eye color*

The classification of eye colors used in anthropological or commercial scales is unsuitable for genetical work. In this context, a better classification is obtained by dividing individuals according to the presence or absence of brown iris pigment. The examination should be made with a magnifying glass and in strong light. Many apparently blue-eyed individuals have small, brown pigment spots. The frequencies of individuals with and without brown iris pigment are shown in Table 9. The highest frequency of brown pigment was found in the mothers, and the lowest in the children. The differences between the fathers and mothers was not significant, while the differences between fathers—children and mothers—children were significant.

All authors since DAVENPORT (1906) agree that the presence of brown pigment is inherited as a dominant trait. Our family data (Table 10) also indicate a dominant inheritance for the brown pigment. No brown-eyed children were found among the offspring in families where both parents lacked brown pigment. Assuming that the absence of brown pigment is a simple Mendelian recessive trait, the gene frequency may be calculated as $\sqrt{0.225}$, or approximately 50 per cent (pooled data for fathers and mothers). With this gene frequency estimate, the expected frequencies of brown-eyed children have been calculated. There is a highly significant lack of brown-eyed children from $(+)\times(+)$ and $(+)\times(-)$ parental combinations ($P<0.001$). This is probably due to the age variation. Many of the children now classified as lacking brown pigment are likely to develop the trait later in life.

The presence or absence of brown iris pigment seems to be of some value in paternity tests. However, more data are needed from families in which both parents lack brown pigment for further empirical verification of the findings that these combinations do not produce children with brown pigment.

The distributions of eye-color types according to the classification of MARTIN-SALLER are found in Table 11. There were certain similarities between the fathers and children (especially for type 1 of the scale). Individuals lacking brown iris pigment belonged mainly to type 1 of this scale. We have not been able to find a correspondence between the types of the eye-color scales and certain hypothetical genotypes as suggested for the MARTIN-SCHULTZ scale by FLEISCHHACKER (1936).

2. *Hair color*

Hair color was primarily classified according to the scale of FISCHER-SALLER. To avoid too many classes, the scale was reduced to six types (cf. Table 12). The distribution of hair-color types according to this scale showed no significant differences between fathers and mothers (cf. Table 13). The difference between parents and children, however, is very marked, which complicates the use of the trait for medico-legal purposes. The frequency of red hair was lower among the adults as compared to the children ($0.02 > P > 0.01$). This could be due to an epistatic effect where the melanin in dark adults covers the red pigment. Similar observations have been made by FISCHER (1930). REED (1952), who made spectrophotometric examinations, found no simple answer to the mode of inheritance of red hair.

In cases of disputed paternity where one of the men has extremely dark (black) hair and the mother and the other men very light hair, this trait might be of some value. However, with the hair colors commonly found in the Swedish population, this trait does not appear very promising.

3. *Hair form*

The form of the scalp hair has been divided into three degrees of curliness, and straight. The distribution of these types is shown in Table 14. It should be emphazised, however, that an arbitrary classification into different degrees of curliness is bound to be more or less biased. The classification into straight and curly hair is clearly more reliable. The family data (Table 15) with the above-mentioned classification show that there are significant variations in the frequency of straight hair among the offspring in different parental combinations. According to DAVENPORT (1906) and FISCHER (1913), different types of curly hair are inherited as a dominant trait. The present data point in the same direction, though there is no evidence for simple, Mendelian inheritance.

4. *Hair whorl*

The occipital hair whorl has been classified into two types; clockwise and counter-clockwise. In some cases, a safe classification was impossible (baldness in men and "double-whorls"). No significant differences were found between fathers and mothers and between parents and children (cf. Table 9).

The clockwise hair whorl has been claimed to be inherited as a dominant trait (cf. SCHADE, 1954). According to v. VERSCHUER (1931) there is no simple Mendelian transmission. Our family data (Table 16) agree with the hypothesis of a simple dominant inheritance. Although there is nothing in the present material to contradict a simple dominant inheritance of the clockwise hair whorl, conclusions concerning the diagnostic value of the trait must be guarded until further confirmations are obtained.

5. *Directions of frontal hair*

KIIL (1948 a) studied the inheritance of frontal hair directions and concluded that the three main types probably are due to combinations of three autosomal alleles. Type I shows downward hair direction on both sides of the forehead, type II downward on one side and upward on the other and type III upward direction on both sides. Twin material showed 100 per cent concordance in monozygotic twins and 43 per cent concordance in dizygotic twins. KIIL (1948 b) also studied the geographical variations of the three types and found that type III had a higher frequency among individuals of south European origin and a lower frequency in individuals of east European extraction. Among 66 mentally deficient individuals (mongoloid idiots), he found no individual of type III, though the expectancy should be 16 or 17 cases.

The frequencies of the three types in the present material are shown in Table 9. There is a significant difference between fathers and mothers for the frequency of type III. There is also a significant difference between fathers and children for type III. Thus in many children type III is not yet developed (cf. KIIL's suggestions concerning the frequency of type III among mentally retarded individuals).

Our family data (Table 17) contradict all of KIIL's conclusions concerning the inheritance of the different types. Although we have followed KIIL's instructions, we like to point out that the classifications are often difficult. Additional family investigations are highly desirable and, at present, this trait should not be used for medico-legal purposes.

6. *Hair between the eyebrows*

The individuals were classified into two types according to the presence or absence of hair between the eyebrows. The frequency of individuals with hair between the eyebrows was higher among men as compared to women ($0.02 > P > 0.01$) and children ($0.05 > P > 0.02$, cf. Table 9).

ROUTIL (1939) and SCHADE (1954) reported that fused eyebrows are inherited as a dominant trait. Our family data (Table 18) indicate that the trait is inherited, but the exact mode of inheritance remains uncertain. Children with hair between the eyebrows may be found in families where both parents lack this trait.

There is a certain risk with this trait, as many adults have the habit of removing these hairs. Due to the observed sex variation and the lack of evidence for simple Mendelian transmission, the diagnostic value of this trait seems relatively low.

7. *Mid-digital hair*

The data on the distribution and inheritance of mid-digital hair have been reported previously (BECKMAN and BÖÖK, 1959). A significant difference between parents and children concerning the presence of mid-digital hair on the ulnar digits was found. Although no simple Mendelian inheritance could be demonstrated, the presence of mid-digital hair showed dominance.

8. DARWIN'*s tubercle*

This trait showed no significant bilateral, sex or age difference (Table 9). Among the fathers and children, the frequency is lower for the left ear, which agrees with earlier results (HILDÉN, 1929). The family data (Table 19) do not indicate that this trait is inherited. QUELPRUD (1935) also concluded that inheritance of DARWIN's tubercle was questionable.

9. *Ear lobes*

The form of the ear lobes was classified according to BRYN (1930). The frequency of clearly attached ear lobes (types V and VI, cf. Table 20) was found to be higher among women, which agrees with the observations reported by SCHADE (1954). The difference between parents and children is striking, the frequency of unattached lobes being higher among the children. According to LEICHER (1928), the unattached ear lobe is inherited as a dominant trait; however, SCHADE (1954) points out that exceptions from the dominant transmission are fairly common. The individuals of the present material were divided into two groups. The family combinations (Table 21 A and B) indicate that the trait is inherited, but not in a simple fashion. The sex and age variations, to-

gether with the uncertainty of transmission, suggest, however, a relatively limited diagnostic value for this trait.

10. *The tongue*

The inheritance of the ability to fold and roll the tongue in different ways has been the object of several investigations. HSU (1948) found that about 3 per cent of the Chinese could fold the distal end of the tongue upwards (without aid of the teeth). He concluded that this ability is probably inherited as a recessive trait. STURTEVANT (1940) found that about 65 per cent of the individuals in a sample from the U.S.A. could roll up the lateral parts of the tongue. No sex-difference was observed.

In the present material, the frequencies of "rollers" and "non-rollers" showed no significant sex or age differences (Table 9). The number of children who could be classified without ambiguity was relatively small in our material due to the difficulties in instructing very small children. According to STURTEVANT (1940), the ability to roll the tongue is inherited as a simple Mendelian trait, although complicating factors may exist. Our family data are consistent with the hypothesis of a dominant transmission (Table 18).

11. *Chin groove*

The chin groove was more common among men (Table 9). The frequency among the children was intermediate between the parents. The frequency for the boys was 52 per cent and for the girls 26 per cent. Apparently there is a sex, but no age, difference. According to PFANNENSTIEL (1951), the chin groove is inherited as a simple dominant trait, which, however, shows some environmental variation. Our family data (Table 23) are consistent with the assumption of a dominant inheritance. However, no simple type of transmission could be found.

12. *Eye openings*

The direction of the eye openings was classified into three types: distally upward directed (U), distally downward directed (D) and horisontal (H). There were no significant differences between the fathers and the mothers, but differences existed between the parents and the children for the types H and U (Table 9). Our family data indicate that the direction of the eye opening might be genetically determined (Table 24). The mode of inheritance, however, remains uncertain.

13. *Folds on the upper eyelids*

No significant difference in the frequency of folds on the upper eyelids could be observed between the fathers and the mothers. The difference between parents and children, however, was significant, the children showing a lower frequency of folds than the adults (Table 9).

According to KEITER (1931) and SCHEIDT (1932), folds on the upper eyelids are inherited as a dominant trait. Our family data (Table 25) point in the same direction. No simple Mendelian inheritance was found.

14. *Hyperextensibility of the proximal thumb joint*

When the proximal thumb joint could be bent backwards considerably more than the usual 90 degrees from the length-axis of the hand, the thumb joint was considered to be hyperextensible. The frequency of hyperextensibility of the proximal thumb joint showed significant bimanual differences for males and females, but not for the children (Table 26). The difference among children, however, goes in the same direction as among the adults. The lower degree of extensibility of the right thumb may depend upon a higher degree of exercise for this hand, which strengthens the muscles and ligaments. The extensibility of the right thumb decreases during growth. The family data (Table 27) showed that the trait is probably inherited, but the mode of inheritance remains uncertain.

15. BONNEVIE's *fingerprint formulae*

BONNEVIE (1927, 1929, and 1931) has studied finger ridges and proposed a genetical mechanism based on three factors (V, R, and U), the so-called BONNEVIE formulae. They have been criticized by many authors (cf. SCHADE, 1954), who have concluded that they are of no practical value.

All individuals in this study have been classified according to BONNEVIE's formulae and the frequencies of "exclusions", assuming simple inheritance, have been calculated. Among 94 families with "undisputed" paternity, 52 "exclusions", of which 13 were double "exclusions" and 3 triple "exclusions", were observed. No differences were found between the three different factors (Table 28). The results confirm that BONNEVIE's formulae should not be used in paternity tests.

16. *Length of the second toe*

ROMANUS (1952 b) has studied the relative length of the second toe and believes that a long second toe is inherited dominantly, but with

reduced penetrance. In the present material, the second toe has been classified into three types: longer than the first toe, length equal to the first toe and shorter than the first toe. No measurements were made. Outlines of the foot were reproduced on a sheet of paper while the investigated individual was sitting. Observations with the individual standing upright with the weight of the body on one foot was found to give varying results depending upon the weight of the individual.

The frequency of a second toe longer than the first toe was significantly lower among the children (cf. Table 29). This does not agree with the observations of ROMANUS who states: "The second toe of younger children is in other words longer relatively more often,". The family data (Table 30 A and B) indicate that the trait may be inherited, but the mode of inheritance remains uncertain.

17. *Tasting*

FOX (1932) discovered that some people were unable to taste phenylthiocarbamide. The ability to taste seems to depend on a dominant gene. There is a sex difference, and some exceptions of inheritance may occur. The percentage of non-tasters varies between different populations. Thus it is about 7 per cent among the Lapps and about 30 per cent in other Europeans (ALLISON and NEVANLINNA, 1952).

The individuals of this investigation have been classified according to the ability to taste a concentrated solution (1300 mg/l) of phenylthiocarbamide. No significant sex or age variations were found (Table 9). The family data (Table 31) showed no incompatibility with the hypothesis of a simple dominant transmission, no tasters being found among the offspring of families where both parents were non-tasters. The expected frequencies have been calculated assuming a frequency of non-tasters=0.36. There is a deficiency of tasters among the offspring of the parental combinations $(+)\times(+)$ and $(+)\times(-)$, indicating a somewhat reduced penetrance for the taster trait.

COMMENTS

The results concerning the qualitative traits have been summarized in Table 32. For two of the seventeen traits no evidence of specific and simple genetical determination was found. Four traits might be inherited in a simple way. Two of these traits (brown eye pigment and tasting) seem to be more reliable, whereas the medico-legal use of the

tongue-rolling and hair whorl traits would result in some ambiguities and practical complications. Monozygotic twins discordant for tongue rolling have been observed (MATLOCK, 1952) and, in some individuals, exercise is important for the development of the trait. The use of hair whorls is complicated by the fact that classification is difficult in some cases.

The purpose of this work was to study the genetics of some traits which have been used for medico-legal purposes and not to construct a test for practical use. Most of the examined traits have failed to show evidence of simple segregation which is necessary for excluding false paternity. Only the presence of brown eye-color pigment and the ability to taste PTC appear to offer such possibilities. There are, however, certain biases connected with these traits, *e.g.* bilateral differences in eye pigmentation and the existence of certain borderline tasters.

The construction of a numerical expression for the probability of paternity rests on an empirical basis. This implies that the statistical significance of the calculated probability of paternity will depend on the size of the sample used as "background population". In other words, the incidence of a useful trait must be determined from sufficiently large and representative samples of the male population as well as from a similar sample of true fathers. Calculations of the probability of paternity based on blood group determinations have an advantage in so far as blood group data are easily available in most populations. For other traits, only very small samples are available and much more family and population data are needed in order to investigate whether or not a reliable empirical method of calculating the probability of paternity can be developed. Furthermore, the frequencies of the useful traits in the population must be thoroughly investigated to settle questions concerning regional variations.

In view of the present rapid development in blood group, serum protein and biochemical genetics which most likely will reveal more traits with simple segregation, adding to those already known, the economy of large anthropological genetical investigations appears slightly dubious unless their primary object is to add to our knowledge of normal variation in man.

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SUMMARY

(1) The genetics of 25 quantitative and qualitative traits which are used for so-called anthropological genetical paternity tests have been studied. Data from one hundred families were used. Each family had one child of the age at which these tests are commonly performed (about 3 years).

(2) Large sex, age or bilateral variations were found for eight quantitative traits, which reduces their practical value. The correlations between fathers and children were generally low, but significant on the five per cent level for seven out of eight metrical traits. The highest correlation was found for the total finger ridge count.

(3) For four out of 17 qualitative traits, the present data do not contradict a simple mode of inheritance. Eleven of the traits appear to be inherited, but the type of segregation is obscure. Two of the traits did not seem to be inherited at all. We have not been able to confirm the three allele hypothesis for the inheritance of frontal hair direction as suggested by KIIL (1948).

(4) While the present family material in its specified context might be sufficient for a genetical test of the traits, it is too scanty to be used as a basis for the development of empirical formulae applicable in legal medicine. As only a few traits seem to follow a simple type of inheritance, "exclusions" according to the classical method are rarely to be expected.

To investigate further whether or not an empirical method of calculating the probability of paternity will be useful and practical, many more studies on sufficiently large and representative samples of families and populations are required.

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TABLE 1. *The mean age of the individuals in the present investigation.*

Group	Age in years		No. of ind.
	M	S.D.	
Fathers	32.82	5.6	100
Mothers	30.33	4.6	100
Boys	3.14	0.59	46
Girls	3.04	0.51	54

TABLE 2. *Age distribution of the children.*

Age	Boys	Girls
1.5—1.9	1	0
2.0—2.4	4	6
2.5—2.9	15	26
3.0—3.4	14	9
3.5—3.9	8	11
4.0—4.4	4	2
Total	46	54

TABLE 3. *Distribution of the parents by counties of birth.*

County	Men	Women	Both sexes
Stockholm	14	18	32
Uppsala	28	33	61
Södermanland	2	4	6
Östergötland	2	1	3
Jönköping	0	1	1
Kronoberg	2	0	2
Kalmar	1	2	3
Gotland	2	0	2
Blekinge	1	0	1
Kristianstad	1	5	6
Malmöhus	4	5	9
Halland	1	2	3
Göteborgs och Bohus	1	2	3
Älvsborg	0	0	0
Skaraborg	2	0	2
Värmland	2	3	5
Örebro	2	0	2
Västmanland	7	1	8
Kopparberg	4	5	9
Gävleborg	3	5	8
Västernorrland	2	7	9
Jämtland	4	1	5
Västerbotten	1	1	2
Norrbotten	8	0	8
Outside the country	6	4	10
Total	100	100	200

TABLE 4. *Differences between the fathers and the mothers. Quantitative traits.*

Character	Fathers			Mothers			Difference	
	n	M	S. D.	n	M	S. D.	t	P
Cephalic index	100	78.0	3.14	100	78.8	2.71	1.95	≈ 0.05
Nose-lip index	100	69.9	2.75	100	71.2	3.05	2.93	$0.01 > P > 0.001$
Interocular biorbital index	100	372.2	28.8	100	364.1	24.4	2.18	$0.05 > P > 0.02$
Bi-acromial diam. / Inter-iliocristal breadth	100	1.30	0.11	96	1.27	0.08	2.28	$0.05 > P > 0.02$
Extensibility of the distal thumb joint-degrees	100	34.8	14.8	100	34.9	13.7	0.07	$P > 0.9$
Ear index	100	53.5	3.67	100	52.5	3.92	2.72	$0.01 > P > 0.001$
Ear angle-degrees	99	24.8	7.9	100	13.9	5.6	11.35	$0.001 > P$

TABLE 5. *Differences between the children and the fathers. Quantitative traits.*

Character	n	M		Difference children-fathers			
		Children	Fathers	M	S. D.	t	P
Cephalic index	100	81.0	78.0	+ 3.0	4.62	6.49	$0.001 > P$
Nose-lip index	100	69.4	69.9	− 0.5	3.98	1.26	$0.3 > P > 0.2$
Interocular biorbital index	100	333.8	372.2	− 38.4	29.92	12.83	$0.001 > P$
Bi-acromial diam. / Inter-iliocristal breadth	95	1.30	1.30	± 0.0	0.12	0.00	1.00
Extensibility of the distal thumb joint-degrees	100	29.0	34.8	− 5.8	15.89	3.65	$0.001 > P$
Ear index	100	59.0	53.5	+ 5.5	4.09	13.45	$0.001 > P$
Ear angle-degrees	99	20.6	24.8	− 4.2	9.99	4.18	$0.001 > P$

TABLE 6. *Differences between the children and the mothers. Quantitative traits.*

Character	n	M		Difference children-mothers			
		Children	Mothers	M	S. D.	t	P
Cephalic index	100	81.0	78.8	+ 2.2	4.72	4.66	0.001 > P
Nose-lip index	100	69.4	71.2	− 1.8	4.32	4.17	0.001 > P
Interocular biorbital index	100	333.8	364.1	− 30.3	27.66	10.95	0.001 > P
Bi-acromial diam. / Inter-iliocristal breadth	95	1.30	1.27	+ 0.03	0.10	2.92	0.01 > P > 0.001
Extensibility of the distal thumb joint-degrees	100	29.0	34.9	− 5.9	15.68	3.76	0.001 > P
Ear index	100	59.0	52.5	+ 6.5	4.56	14.25	0.001 > P
Ear angle-degrees	99	20.6	13.9	+ 6.7	9.07	7.35	0.001 > P

TABLE 7. *Differences between the right (R) and the left (L) side. Quantitative traits.*

Character		n	R	L	Difference R—L			
					M	S. D.	t	P
Extensibility of the distal thumb joint-degrees	Fathers	100	34.1	35.5	− 1.4	7.72	1.81	0.1 > P > 0.05
	Mothers	100	33.7	36.2	− 2.5	5.64	4.43	0.001 > P
	Children	100	28.1	29.9	− 1.8	6.50	2.77	0.01 > P > 0.001
Ear index	Fathers	100	52.8	54.1	− 1.3	2.42	5.37	0.001 > P
	Mothers	100	52.4	52.6	− 0.2	2.64	0.76	0.5 > P > 0.4
	Children	100	58.4	59.6	− 1.2	2.88	4.17	0.001 > P
Ear angle	Fathers	99	24.6	25.0	− 0.4	4.70	0.85	0.4 > P > 0.3
	Mothers	99	14.0	13.8	+ 0.2	2.92	0.68	0.5 > P > 0.4
	Children	99	20.4	20.8	− 0.4	5.28	0.75	0.5 > P > 0.4

TABLE 8. *Correlations between the fathers and the children. Quantitative traits.*

Character	Correlation between		
	fathers—sons	fathers—daughters	fathers--children
Cephalic index	+0.25±0.14	+0.09±0.13	+0.17±0.10
Bi-acromial diameter × 100 / Inter-iliocristal breadth	+0.22±0.14	+0.24±0.13	+0.23±0.10
Interocular-biorbital index	+0.34±0.13	+0.45±0.11	+0.34±0.09
Nose-lip index	+0.20±0.14	+0.35±0.12	+0.28±0.09
Ear index	+0.36±0.13	+0.36±0.12	+0.35±0.09
Ear angle	+0.32±0.14	+0.18±0.13	+0.25±0.09
Extensibility of the distal thumb joint	+0.19±0.14	+0.34±0.12	+0.28±0.09
Total finger ridge count (BÖÖK, 1957)	+0.51±0.11	+0.38±0.12	+0.43±0.08

TABLE 9. *The frequencies of twelve qualitative traits among the parents and the children.*

Traits	Fathers		Mothers		Children	
	n	%	n	%	n	%
Brown eye color pigment	100	72.0	100	83.0	100	48.0
Red hair color	100	1.0	98	1.0	98	8.2
Curly hair	98	58.2	98	54.1	98	46.9
Clockwise hair-whorl	91	85.7	92	84.8	99	82.8
Direction of frontal hair						
Type I	92	34.8	97	52.6	89	57.3
„ II	92	21.7	97	19.6	89	23.6
„ III	92	43.5	97	27.8	89	19.1
Hair between the eyebrows	100	84.0	100	70.0	100	72.0
DARWIN's tubercle						
Right	100	18.0	100	12.0	100	19.0
Left	100	11.0	100	13.0	100	11.0
Tongue rolling	100	71.0	100	74.0	22	77.3
Chin groove	100	50.0	100	24.0	100	38.0
Direction of eye openings						
Horizontal	100	29.0	100	30.0	100	17.0
Distally downwards	100	4.0	100	2.0	100	0.0
Distally upwards	100	67.0	100	68.0	100	83.0
Folds on the upper eyelids	100	28.0	100	22.0	100	9.0
Ability to taste P. T. C.	100	63.0	100	64.0	100	58.0

TABLE 10. *Brown eye color pigments. Parental combinations.*

Parents		Children		Total
		(+)	(−)	
(+)×(+)	obs.	37	25	62
	exp.	58.5	3.5	
(+)×(−)	obs.	11	20	31
	exp.	23.7	7.3	
(−)×(−)	obs.	0	7	7
	exp.	0	7	
Total		48	52	100

(+)=Brown pigment.

TABLE 11. *Frequencies of eye color types according to* MARTIN-SALLER*'s classification.*

	Eye color types								
	1	2	3	4	5	6	7	8	Total
Fathers	59	2	15	7	8	2	6	1	100
Mothers	35	9	24	6	7	6	5	8	100
Children	61	4	9	6	6	2	9	3	100
Total	155	15	48	19	21	10	20	12	300

TABLE 12. *Classification of hair color.*

Nr	Present classification color	Classification after FISCHER-SALLER
1	Black	X, Y
2	Brownish black	U, V, W
3	Medium brown	K, L, M, N, O, P, Q, R, S, T
4	Light brown	G, H, J
5	Flaxen	A, B, C, D, E, F
6	Red	I, II, III, IV, V, VI

TABLE 13. *Frequencies of hair color types according to the classification described in Table 12.*

	1	2	3	4	5	6	Total
Fathers	1	19	63	10	6	1	100
Mothers	0	23	65	4	5	1	98
Children	0	1	25	11	53	8	98
Total	1	43	153	25	64	10	296

TABLE 14. *Distribution of different types of head-hair form.*

0=straight hair, 1—3=increasing degree of curliness.

	3	2	1	0	Total
Fathers	11	19	27	41	98
Mothers	6	13	34	45	98
Children	12	2	32	52	98
Total	29	34	93	138	294

Difference parents-children: $\chi^2=11.05$, 3 d. f., $0.02 > P > 0.01$.

TABLE 15. *Curly hair. Parental combinations.*

(+)=curly hair, (—)=straight hair.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	20	13	33
(+)×(—)	22	22	44
(—)×(—)	4	17	21
Total	46	52	98

Heterogeneity: $\chi^2=15.06$, 2 d. f., $P < 0.001$.

TABLE 16. *Hair whorl. Parental combinations.*

(+)=clockwise hair whorl, (—)=counter-clockwise hair whorl.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	46	4	50
(+)×(—)	13	6	19
(—)×(—)	0	3	3
Total	59	13	72

TABLE 17. *Directions of frontal hair. Parental combinations.*
N=not distinct.

Father	Mother	Children				
		I	II	III	N	Total
I	I	13	3	2	2	20
II	II	1	2			3
III	III	9	2	3	1	15
I	II	5				5
I	III	2	2	1	1	6
II	I	4	6	1	1	12
II	III	2	1	1		4
III	I	11	1	3	1	16
III	II	2	3	3	1	9
N	N				1	1
N	I	1	1		1	3
N	II				2	2
N	III			2		2
I	N	1				1
II	N			1		1
III	N					0
Total		51	21	17	11	100

TABLE 18. *Hair between the eyebrows. Parental combinations.*
(+)=presence of hair between the eyebrows. (—)=absence of hair between the eyebrows.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	50	11	61
(+)×(—)	20	12	32
(—)×(—)	2	5	7
Total	72	28	100

TABLE 19. DARWIN's *tubercle. Parental combinations.*
(+)=presence of DARWIN's tubercle on one or both ears,
(—)=absence of DARWIN's tubercle.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	1	3	4
(+)×(—)	10	28	38
(—)×(—)	14	44	58
Total	25	75	100

TABLE 20. *Frequencies of different types of ear lobes. Type I is completely unattached. Types II—VI different degrees of attachment (see text).*

	Type of ear lobes						Total
	I	II	III	IV	V	VI	
Fathers:							
Right	24	33	22	19	1	1	100
Left	24	34	19	20	1	2	100
Mothers:							
Right	16	38	16	21	4	5	100
Left	17	35	19	21	3	5	100
Children:							
Right	31	61	3	5	0	0	100
Left	31	60	4	5	0	0	100

TABLE 21. *Ear lobes. Parental combinations.*

(+) = unattached ear lobe (corresponding to types I and II), (—) = attached ear lobe (corresponding to types III—VI).

A.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	31	0	31
(+)×(—)	47	4	51
(—)×(—)	15	3	18
Total	93	7	100

(+) = unattached ear lobe (corresponding to type I), (—) = attached ear lobe (corresponding to types II—VI).

B.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	3	0	3
(+)×(—)	16	15	31
(—)×(—)	10	56	66
Total	29	71	100

TABLE 22. *Tongue rolling. Parental combinations.*

(+) = ability to roll the tongue, (—) = unability to roll the tongue.

Parents	Children		Total
	(+)	(−)	
(+)×(+)	10	2	12
(+)×(−)	7	2	9
(−)×(−)	0	1	1
Total	17	5	22

TABLE 23. *Chin groove. Parental combinations.*

(+) = chin groove present, (—) = chin groove absent.

Parents	Children		Total
	(+)	(−)	
(+)×(+)	10	1	11
(+)×(−)	24	28	52
(−)×(−)	4	33	37
Total	38	62	100

TABLE 24. *Direction of eye openings. Parental combinations.*

(+) = upward direction, (—) = horizontal or downward direction.

Parents	Children		Total
	(+)	(−)	
(+)×(+)	44	4	48
(+)×(−)	32	8	40
(−)×(−)	7	5	12
Total	83	17	100

TABLE 25. *Folds on the upper eyelids. Parental combinations.*

(+) = presence of folds, (—) = absence of folds.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	2	4	6
(+)×(—)	6	32	38
(—)×(—)	1	55	56
Total	9	91	100

TABLE 26. *Extensibility of the proximal thumb joint.*

(+) = hyperextensibility.

	(+)	(—)	Total
Fathers:			
Right	9	91	100
Left	25	75	100
Mothers:			
Right	8	92	100
Left	24	76	100
Children:			
Right	20	80	100
Left	24	76	100

Bimanual difference: Fathers: $\chi^2=9.14$, 1 d.f., $0.01>P>0.001$.
Mothers: $\chi^2=9.55$, 1 d.f., $0.01>P>0.001$.

Difference (right thumb): Fathers—Children: $\chi^2=4.88$, 1 d.f., $0.05>P>0.02$.
Mothers—Children: $\chi^2=6.00$, 1 d.f., $0.02>P>0.01$.

TABLE 27. *Extensibility of the proximal thumb joint. Parental combinations.*

(+) = hyperextensibility of one or both joints.

Parents	Children		Total
	(+)	(—)	
(+)×(+)	4	3	7
(+)×(—)	17	18	35
(—)×(—)	8	50	58
Total	29	71	100

TABLE 28. *Distributions of exclusions according to* BONNEVIE's *formulae.*

»Exclusion» of	Exclusion by factor			Total
	V	R	U	
Fathers	6	4	11	21
Mothers	10	6	6	22
Fathers or mothers	7	14	4	25
Fathers and mothers	1	0	2	3
Total	24	24	23	71

Frequencies of multiple exclusions.

	Type				Total
	VR	VU	RU	VRU	
»Exclusions»	2	8	3	3	16

TABLE 29. *Frequencies of different types of second toe.*

1=second toe shorter than the first toe, 2=second toe of length equal to the first toe, 3=second toe longer than the first toe.

	1	2	3	Total
Fathers:				
Right	75	10	15	100
Left	69	14	17	100
Mothers:				
Right	80	10	10	100
Left	80	6	14	100
Children:				
Right	88	9	3	100
Left	81	14	5	100

TABLE 30. *Length of the second toe. Parental combinations.*

L=longer or equally long (nos. 2 and 3), S=shorter.

A. *Right foot.*

Parents	Children		Total
	L	S	
L×L	3	1	4
L×S	6	31	37
S×S	3	56	59
Total	12	88	100

B. *Left foot.*

Parents	Children		Total
	L	S	
L×L	4	1	5
L×S	11	30	41
S×S	4	50	54
Total	19	81	100

TABLE 31. *P.T.C. tasting. Parental combinations.*

(+)=tasters, (—)=non-tasters.

Parents		Children		Total
		(+)	(—)	
(+)×(+)	obs.	35	8	43
	exp.	39	4	
(+)×(—)	obs.	23	18	41
	exp.	29	12	
(—)×(—)	obs.	0	16	16
	exp.	0	16	
Total		58	42	100

TABLE 32. *Qualitative characters. Summary of the results of the analysis of the family data.*

Character	Nothing contradicts simple inheritance	Inherited though not in a simple way	Sex variation	Age variation	Bilateral variation
Eye color	+		−	+	
Hair color	−	+	−	+	
„ form	−	+	−	+	
„ whorl	+		−	−	
Frontal hair	−	+	+	+	
Hair between the eyebrows	−	+	+	+	
Mid-digital hair	−	+	−	+	−
DARWIN's tubercle	−	−	−	−	−
Ear lobes	−	+	+	+	−
Tongue-rolling	+		−	−	
Chin groove	−	+	+	+	
Eye openings	−	+	−	+	
Folds on the upper eyelids	−	+	−	+	−
Extensibility of the proximal thumb joint	−	+	−	+	+
BONNEVIE's fingerprint formulae	−	−	−	−	
Length of the second toe	−	+	−	+	−
Tasting	+		−	−	

ASYNAPSIS IN SORGHUM

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I. INTRODUCTION

THE widespread occurrence of unpaired chromosome strands at early prophase of meiosis, asynapsis or desynapsis, has previously been noted in many species including *Sorghum,* for example *Zea* (BEADLE, 1933), *Secale* (PRAKKEN, 1943), *Picea* (ANDERSSON, 1947), *Sorghum* (HUSKINS and SMITH, 1934; KRISHNASWAMY and MEENAKSHI, 1957). Such a mutant has been found in the sorghum variety Experimental 3 which has been studied extensively from the standpoint of the origin of true-breeding mutants after colchicine treatment (FRANZKE and ROSS, 1952; HARPSTEAD *et al.*, 1954; ROSS *et al.*, 1954). Although the mutant was noted during mutation experiments involving the use of colchicine, control seed from the same plant was found to contain the mutation so that its occurrence did not result from colchicine treatment. This study deals with the inheritance and cytological behaviour of this mutant. Since there was no evidence that complete synapsis had occurred at the early stages of pachynema, the term "desynapsis" (LI *et al.*, 1945) is not used.

Material and methods

Plants studied were grown from selfed seed produced in the greenhouse from a single plant of Experimental 3, a sorghum variety described previously (FRANZKE and ROSS, 1952). This variety has been propagated by selfing for 14 generations and is true-breeding. Plants and their progenies from three lots of seed representing each of three panicles from the above plant were studied.

For the study of meiosis, inflorescences were fixed in 3 ethyl alcohol: 1 glacial acetic acid and stored in 70 % ethyl alcohol. The chromosomes were stained with propionic carmine.

TABLE 1. *Progenies of plants from 8 seeds from panicle no. 1 illustrating segregation of fertile and sterile plants.*
The other 27 plants were fertile and produced fertile progenies.

Parent plant	Progeny			
	Fertile plants		Sterile plants	
	Number	% fertility[1]	Number	% fertility[1]
Sterile	0	—	40±	8.7
»	0	—	40±	8.8
»	0	—	40±	3.2
Fertile (segregating)	36	60.3	13	3.7
» »	33	66.2	9	10.4
» »	32	55.6	9	8.2
Fertile (nonsegregating)	40±	73.3	0	—
» »	40±	56.2	0	—

[1] Mean of five panicles, each from a separate plant.

II. RESULTS

1. Inheritance

Progeny tests of 35 sorghum plants, all from the same selfed panicle, (29 had been treated with colchicine as seedlings and 6 were untreated controls) gave 29 progenies of high fertility, 3 segregating in an approximate 3 fertile: 1 sterile ratio, and 3 with all plants of low fertility as shown in Table 1. Fertility ranged from 55.6—73.3 % among the fertile plants and from 3.2—10.4 % among the sterile plants. This level of fertility of the fertile plants is not abnormal in this line though no chromosomal irregularity has been detected (HARPSTEAD *et al.*, 1954). Since the plants containing the mutant gene causing sterility occurred

TABLE 2. *Comparison of proportions of fertile and sterile plants from selfed seed of three different panicles from the same sorghum plant of variety Experimental 3.*

Panicle no.	Progeny	
	Number of fertile plants	Number of sterile plants
1	32	3
2	27	13
3	41	0

in the colchicine-treated group, it might have been possible that the mutations had resulted from treatment as reported by FRANZKE and ROSS (1952) and ROSS *et al.* (1954), except for the consideration that the odds for the same mutation occurring simultaneously in six different plants are very low indeed. Therefore, the alternative possibility of the occurrence of a chimeral sector in the original plant carrying a natural mutation causing sterility was investigated by progeny testing selfed seed from two other panicles from the same plant. As shown in Table 2, one of these segregated 27 fertile: 13 sterile, while all 41 plants of the other progeny were fertile. It would seem that panicle no. 1 was a chimera with a sector carrying the mutated gene in the heterozygous condition, that panicle no. 2 was probably made up wholly of tissue heterozygous for the mutated gene, while panicle no. 3 did not carry it. A mutation for asynapsis, therefore, appears to have occurred during the development of this plant.

2. Meiosis in the fertile plants

An examination of the meiotic behaviour of chromosomes in fertile plants was made for comparison with previous cytological studies of Experimental 3 (HARPSTEAD *et al.*, 1954). Sorghum chromosomes at prophase of meiosis have very definite heterochromatic regions on either side of the centromere and very lightly staining ends as shown at the pachytene stage in Fig. 1 and at diakinesis in Fig. 2. The centromeres are medianly or submedianly located and the nucleolus is also found in a submedian position. At pachytene the chromosomes were quite regularly paired throughout the whole of the heterochromatic region, but lack of pairing in the euchromatic chromosome ends was sometimes observed (Fig. 1). A split end is seen at 10:00 o'clock, and unpaired strands are visible at 9:00 o'clock. Considerable variability in the degree of this lack of pairing was observed between cells, but as shown in Figs. 2, 3 and 4, bivalent formation and division of bivalents is regular. Examination of at least 100 cells at each of the following stages, diakinesis, metaphase I, anaphase I, metaphase II, anaphase II and quartet showed no irregularities. Some variation in the chiasmata frequency may be presumed, since at diakinesis one or, in a very few cases, two open bivalents were observed in 20—25 % of the cells examined. In no case, however, was any irregularity in division observed, indicating that the unpaired condition sometimes observed in the chromosome ends at pachynema does not influence the distribution of chro-

mosomes. This agrees with observations of HARPSTEAD *et al.* (1954) who examined meiosis in plants of this variety of sorghum without the gene for asynapsis.

In some instances, slight stickiness between bivalents was observed at diakinesis, but at metaphase bivalents were not observed to be associated. Some connections between chromosomes were also observed at anaphase, but again these do not appear to have significance from the standpoint of affecting segregation.

3. Meiosis in Sterile Plants

Division I

Examination of each of the stages of meiosis was made in sterile plants of each of the 6 progenies from panicle 1 showing sterility. Unpaired sections involving the heterochromatic regions and the centromeres were found in abundance in pachytene stages of all sterile plants regardless of whether they occurred in segregating or nonsegregating progenies. Fig. 5 illustrates this general lack of pairing and also the presence of nonhomologous pairing which sometimes though not commonly occurs. The unpaired regions of homologous chromosomes with one of the two on each side of another chromosome pair as shown by the arrow at 10:00 o'clock indicate that pairing had not occurred in this region, otherwise the paired chromosome would have been excluded from this position. Observations made on normal pachytene stages showed no such configuration. Because of this observation and since earlier stages without unpaired chromosomes were not detected, the term asynapsis is considered more proper than desynapsis in describing the phenomenon.

This lack of pairing results in variable numbers of univalents at later stages as shown in Figs. 6—8. Four univalents are shown at early diakinesis (Fig. 6), 8 at late diakinesis (Fig. 7), 4 at metaphase (Fig. 8). This variability was examined further (Table 3). The number of univalents did not appear to increase from diakinesis to metaphase, but the number of open bivalents may have increased somewhat indicating that some chiasmata may completely terminalize as metaphase is approached. The largest number of univalents noted in any cell was 12, the range extending from 0 upwards. No marked difference between asynaptic plants in number of univalents or of open bivalents was noted. The presence of open bivalents may be expected to cause pre-

TABLE 3. *Numbers of I's and open II's at diakinesis and metaphase in asynaptic sorghum plants.*

Plant No.	Stage	No. of P. M. C.'s	I's per P. M. C.		Open II's per P. M. C.	
			Mean	Range	Mean	Range
1	Diakinesis	16	4.4±2.8	0—10	—	—
2	»	46	3.1±1.7	0—8	3.2±1.3	1—7
3	»	14	3.0±2.5	0—10	3.7±1.2	1—5
1	Metaphase	16	4.8±2.8	2—10	4.0±1.8	1—7
2	»	40	2.8±1.8	0—8	4.5±2.2	1—8
3	»	94	3.4±2.4	0—12	4.3±1.6	0—9

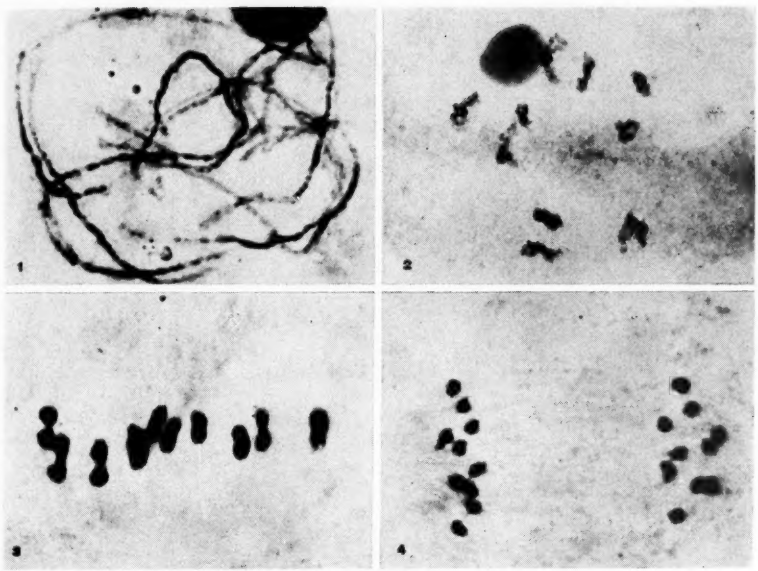
cocious separation as is shown in Fig. 8. The univalents were characteristically found at the outside of the spindle mechanism as shown in this figure where two are at the extreme left and two at the extreme right.

A variable number of laggards was observed at the equator after separation of the bivalents (Figs. 9 and 10). This is further illustrated by data taken from observations made at anaphase I of plant 3 as shown below.

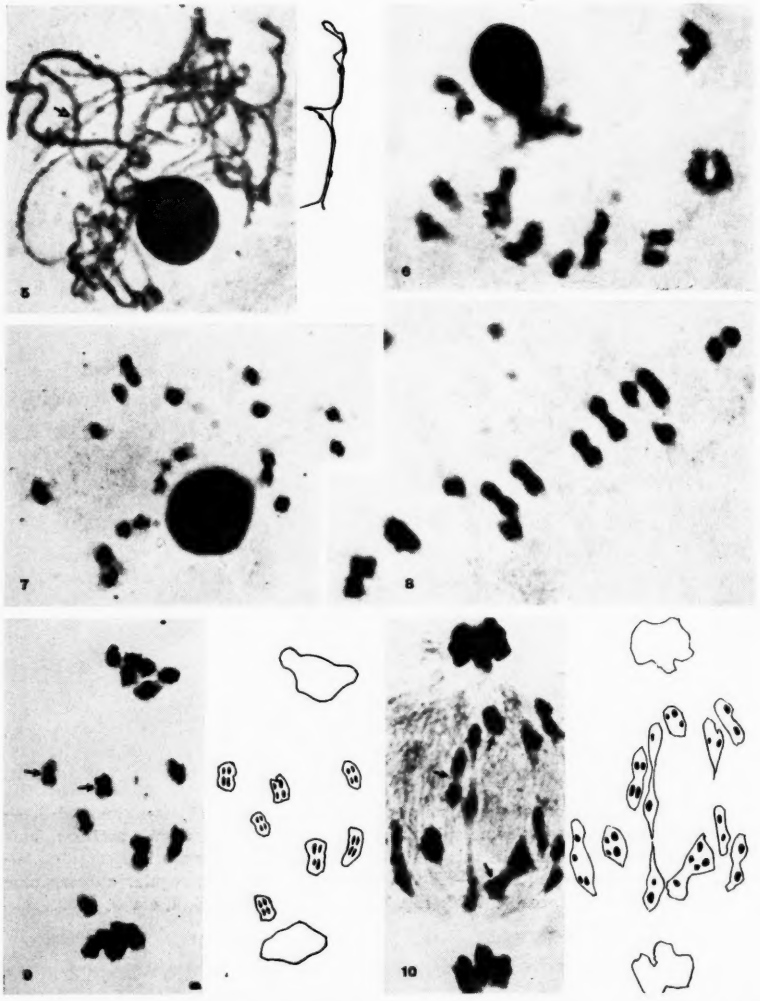
Stage	Plant No.	Number of cells	Laggards per cell	
			Mean	Range
Anaphase I	3	62	2.7±2.5	0—8

The slight decrease in mean number of laggards in comparison to the number of univalents noted at diakinesis and metaphase may result from the inclusion of univalents with the polar groups.

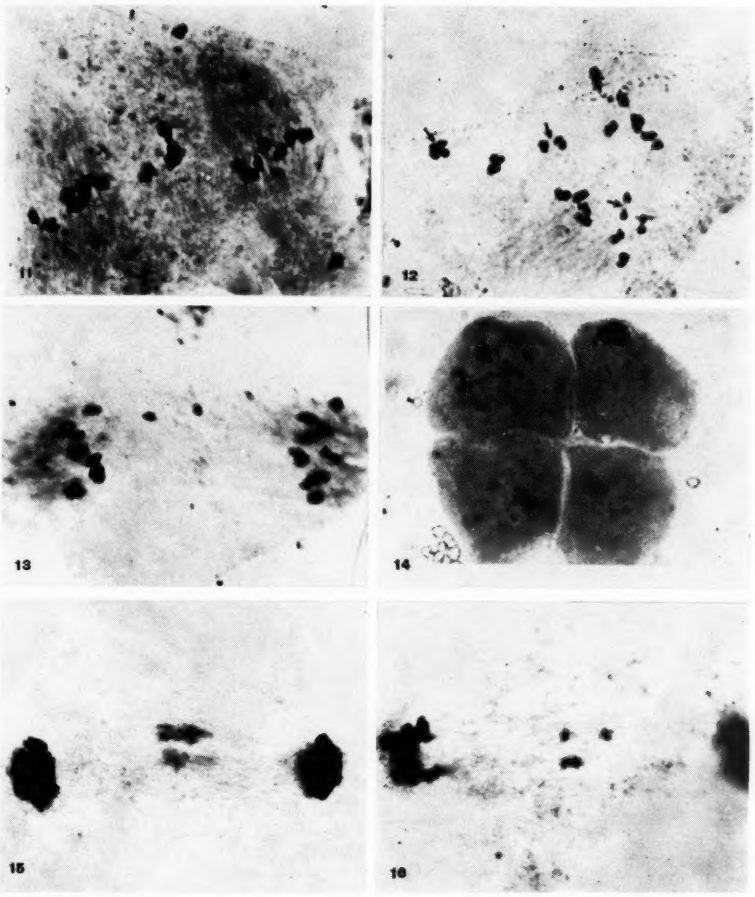
When the chromosomes separate at anaphase I, univalents are usually left at the plate where a neocentric action develops which pulls the chromosome ends poleward, stretching the chromosome across the center of the cell. The beginning of this phenomenon is shown in Fig. 9 where the chromosome ends are beginning to be drawn towards the poles. In Fig. 10, the stress set up by the poleward pull is very evident. Since the centric region is not functionally divided at this point, a stretching occurs as shown in Fig. 10 where the stress has been sufficient to tear some of the univalents apart. Depending upon how the univalent is oriented, the division may occur between the chromatids (as indicated by the arrow in the center of Fig. 9) or transversely at the centromere. An example of a possible transverse division is indicated



Figs. 1—4. First division of meiosis in a fertile sorghum plant, Experimental 3. — Fig. 1. Pachynema showing some split ends and unpaired euchromatic sections. This condition sometimes occurs in this material. — Fig. 2. Diakinesis showing 10_{II}. — Fig. 3. Metaphase showing 10_{II}. — Fig. 4. Anaphase I showing regular chromosome division. — Fig. 1, c:a × 1400; Fig. 2, c:a × 1000; Figs. 3, 4, c:a × 1100.



Figs. 5—10. First division of meiosis of an asynaptic sorghum plant, Experimental 3. — Fig. 5. Pachynema showing large numbers of unpaired regions and nonhomologous pairing on the right. — Fig. 6. Early diakinesis showing $8_{II}+4_{I}$. — Fig. 7. Late diakinesis showing $6_{II}+8_{I}$. — Fig. 8. Metaphase I showing 8_{II} including 2 precociously separating and 4_{I}. — Fig. 9. Late anaphase I with 6_{I} beginning to show neocentric activity as the chromosome arms are pulled towards the poles. On the extreme left center, longitudinal orientation of one chromosome is shown; in the other chromosomes, transverse orientation is indicated. — Fig. 10. Anaphase I showing 8_{I} being pulled apart by neocentric action on the chromosome arms. — Figs. 5, 6, 8, 9, c:a × 1400; Fig. 7, c:a × 1250; Fig. 10, c:a × 1520.



Figs. 11—14. Second division of meiosis in sterile plants. — Fig. 11. Metaphase II showing univalents outside the spindles. These were observed to have spindle fibers attached to the centromere region. — Fig. 12. Anaphase II. Arrows indicate an undivided univalent on the left, a divided univalent in the center and a half univalent torn at the centromere region on the right. — Fig. 13. Fragments of a half univalent, torn apart at the centromere region by neocentric action, proceed towards opposite poles. — Fig. 14. A quartet showing diffuse nucleoli in the two spores at the left, and micronuclei near inner spore walls. Figs. 15—16. First and second anaphase of meiosis of tetraploid sorghum, Experimental 3. — Fig. 15. Anaphase I showing univalents being subjected to neocentric action at equatorial region. — Fig. 16. Anaphase II showing one half univalent pulled apart at centromere region and the parts proceeding poleward. — Figs. 11—13, c:a × 1100; Fig. 14, c:a × 850; Figs. 15—16, c:a × 1400.

by the arrow at the left of Fig. 9 where the two large arms of the chromosome are at the bottom and the two short arms at the top. The drawing of the chromosomes to the right of the photograph illustrates this more clearly. A similar case is shown by the arrow near the center in Fig. 10, where the beginning of a separation of the two short arms from the two long arms is seen. This could result in isochromosomes. The possibility of a telocentric chromosome being formed is indicated by the two arrows further to the right where a long arm has been torn from a univalent. As illustrated in the drawing, one long arm and two short arms are shown above and the other long arm below, now connected to a univalent probably by means of matrix material.

Division II

The most obvious irregularity noted at the second division is the disposition of the undivided univalents which are seen outside the spindle mechanism at metaphase II (Fig. 11). Each of these univalents was observed to form an individual spindle quite apart from the main spindle apparatus, but it was not effective in pulling the chromatids apart as seen at anaphase II at the arrow on the left in Fig. 12. In one instance a univalent (indicated by the center arrow) has evidently been included in the spindle and is belatedly dividing. In other cases, half univalents which have already been pulled apart at first anaphase divide transversely. Divisions of half univalents, shown by the arrow at the right in Fig. 12 and also in Fig. 13, result in breakage at the centromere region. This appears to be another instance of neocentric action. One chromosome arm goes to each pole as indicated in Fig. 13 and may be included within the nuclear membrane. Therefore, the number of chromosomes quite often varies between nuclei of the quartet, and micronuclei occur as shown in Fig. 14. Another result, also illustrated in Fig. 14, is the formation of diffuse nucleoli as a consequence of the nucleolar bearing chromosomes forming univalents at prophase. The diffuse nucleoli are the congregated bodies in the two spores on the left hand side of the quartet, while the micronuclei are found near the inner cell walls. An estimate of the occurrence of spores with single, two and diffuse nucleoli is shown in Table 4. In three different sterile plants the percentages with diffuse nucleoli were 7.1, 7.6 and 10.7. This is close to the one in ten ratio that would occur according to chance if the nucleolar bearing chromosome were implicated at random in the formation of univalents and excluded from the polar groups. This would indicate that the asynaptic phenomenon is not differential in its

TABLE 4. *The occurrence of spores with single, two and diffuse nucleoli in asynaptic plants of sorghum.*

Plant No.	Number observed	Number of nucleoli per spore			% with diffuse nucleoli
		1	2	Diffuse	
2	242	203	5	34	7.1
3	214	178	8	28	7.6
4	171	152	3	16	10.7

action and that each chromosome pair has an equal chance of being affected.

III. DISCUSSION

1. Nature of asynapsis

HUSKINS and SMITH (1934) observed a case of asynapsis in Dakota Amber Sorgo which might be related to one of the parents involved in the background of variety Experimental 3. The similarity of their description to that of the present asynaptic condition suggests that the same gene may have been mutated in both instances. KRISHNASWAMY and MEENAKSHI (1957) report two cases of "desynapsis" in sorghum which were also inherited as simple Mendelian recessives, one of which had an average of 6.5 and the other of 0.6 bivalents per cell. In both cases, however, some cells were found in which no bivalents were present so that it appears that a greater degree of disassociation occurred in their material. They assigned the symbols as^1 and as^2 to these genes. Since the gene described here has a different effect from either of these in the average number and range of bivalents formed and seems, therefore, to be distinct from them, it is proposed that this gene be designated as^3.

The cause for this phenomenon must be found in the breakdown of the mechanism effecting synapsis and possibly of the mechanism effecting the subsequent separation of the strands after pachynema. There appears also to be some lack of specificity in pairing as shown by the occasional occurrence of nonhomologous pairing. There seems to be good evidence, both from observations of unpaired strands at late zygonema and early pachynema, and from circumstantial evidence derived from the observation of a region which could not have been paired since homologous chromosomes were separated by another chro-

mosome pair, that an abnormal condition at the time of synapsis, rather than lack of chiasmata formation, is responsible for the unpaired condition of the chromosomes. The low number of chiasmata may then be considered a result of, rather than the cause of, lack of pairing.

Various hypotheses concerning chromosome pairing have recently been reviewed by SWANSON (1957). He concludes that the problem of synapsis is still an enigma. Use of the asynaptic gene might afford an experimental approach to this problem since some substance or substances, perhaps enzymatic in nature, which effect pairing of the chromosome might be absent. Means might be devised to ascertain such a difference between the normal and asynaptic conditions. EHRENBERG (1949) also suggested the possibility that the asynaptic condition might result from the lack of an enzyme.

2. Behaviour of univalents

The chromatids of univalents at anaphase I in this variety of sorghum do not separate in normal mitotic fashion as is the usual case in rye (PRAKKEN, 1943). Instead the centromere would seem to be still functionally single. If it is double or quadruple, as had been observed in root tip chromosomes (ÖSTERGREN, 1947; TJIO and LEVAN, 1950) and also at meiosis (LIMA-DE-FARIA, 1956), the parts are held together. ANDERSSON (1947) describes such a misdivision in which the centromere components themselves are attracted to each pole resulting in a stretching of the centromere region. ÖSTERGREN (1951) considers the centromeres to be double structures which establish the position of the univalents by means of their orientation with respect to the poles of the spindle. In the present case, when univalents are left at the equatorial plate after anaphase movement of other chromosomes, neocentric action of a type somewhat similar to that described by RHOADES (1952) takes place, so that the ends are drawn towards the poles and a stretching of the centromere region results. The centromere proper in these cases would seem to play a passive role. ÖSTERGREN and BAJER (1960) have recently detected neocentric movements of chromosomes at mitoses indicating that such action may normally occur during anaphase separation of chromosomes. In the case of lagging univalents described here, neocentric action may take over the whole function of chromosome disjunction as a result of the absence of the force acting on the centromere. Whether the chromosomes are at right angles or parallel to the spindle seems to have no effect on this pulling action. It may be

expected that isochromosomes result from this force, but probably in many instances they would not be included with either nucleus but would form micronuclei and so be lost.

The orientation of the univalent as described by ÖSTERGREN (1951) may determine whether it is included with one of the polar groups of chromosomes at telophase I or remains at the equator. The number of laggards at the equator may, therefore, be expected to be lower than the number of univalents at metaphase I.

The univalents which become a part of the polar group would be expected to arrange themselves at the equatorial region at metaphase II and divide normally at anaphase. The already divided univalents may be fractured at this division and would, if included in a nucleus, form telocentric chromosomes or isochromosomes. Telocentric or fractured chromosomes should form a heteromorphic pair at prophase of meiosis when associated with the normal homologue (BROWN 1958) and should be easily identified. A marked chromosome of this type would be valuable from the standpoint of testing the hypothesis of somatic reduction advanced by FRANZKE and ROSS (1952) and ROSS *et al.* (1954) to explain the homozygous nature of mutants observed after colchicine treatment of the sorghum variety, Experimental 3.

Since RHOADES and VILKOMERSON (1942) have shown that neocentric activity in *Zea* was caused by a heterochromatic region attached to chromosome 10, it was thought that in the present case the gene for asynapsis might also be responsible for this neocentric action. To test this possibility, examination was made of the behaviour of univalents at anaphase I and II in a tetraploid plant of the same variety which did not contain the gene for asynapsis. As shown in Figs. 15 and 16, the univalents found in such tetraploid cells were subject to the same neocentric action as observed in the asynaptic plants. It may therefore be presumed that the phenomenon is not related to asynapsis but that it normally occurs in the variety Experimental 3.

At anaphase II the univalents in the undivided condition lying outside the spindle mechanism were seen to form a spindle of their own, at least spindle fibers could be seen attached to the centromere region. There appeared to be no traction involved in these instances, and the chromatids were not separated. This observation seems to indicate that the spindle mechanism in its origin is intimately connected to the centromere region. The actual traction force, however, seems to require the concerted effect of a number of chromosomes.

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SUMMARY

A mutation for asynapsis, as^a, inherited as a single Mendelian recessive, occurred as a chimeral sector in a sorghum plant of variety Experimental 3. Large regions of pachytene chromosomes were unpaired, and evidence indicated that complete pairing had not occurred. Univalents, which varied in number from 0—12, were found to lag at anaphase I and to be subsequently pulled apart by neocentric action. Transverse or longitudinal separation at the centromere resulted, depending on the orientation of the univalent at the equator. Univalents in which chromatids divided at first division were in certain instances torn apart at anaphase II to form fragments. If cytologically marked chromosomes were obtained from asynaptic material, they would be valuable for testing the theory of somatic reduction after colchicine treatment. The gene for asynapsis may also be valuable as a tool in studying the mechanism of synapsis.

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CHROMOSOMAL EVOLUTION IN THE CICADELLIDAE

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INTRODUCTION

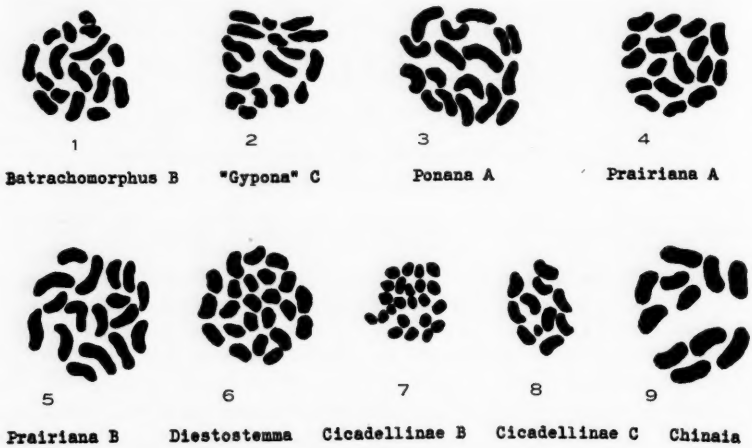
SINCE the publication of a list of chromosome numbers by HALKKA (1959), further contributions to the chromosome cytology of the auchenorrhynchous *Homoptera* have been made by at least MENON (1959), SESHACHAR et al. (1959) and HALKKA (1960). MENON's paper adds to the list one chromosome number determination in the *Membracidae* and HALKKA's one in the *Cicadellidae*.

The present study is an addition to the Cicadellid section of the list. The specimens studied were collected during a trip made by the author to Canal Zone, Panama. Altogether, 25 species representing 6 different subfamilies have been included. The Neotropical zoogeographical region is particularly rich in leafhoppers, and the present sample of karyotypes represents only a small fraction of the Panamanian *Cicadellidae*. In spite of its limitations, the sample affords an interesting comparison with a much larger sample collected previously by a number of authors in the Palearctic and Nearctic regions.

Material and methods

The material for this study was collected in Barro Colorado Island and the adjoining mainland (Frijoles), Canal Zone, Panama, during a 12-day trip in early May, 1959. The following list includes all the individuals in which the chromosome number was determined. In the list the name of each species is followed by the number of specimens.

Batrachomorphus (*Stragania*) n. sp. A 5, *Batrachomorphus* (*Stragania*) n. sp. B 1, *Agallia modesta* 2, *Gypona* sp. A 1, *Gypona* sp. B 2, "*Gypona* sp." C 6, *Ponana* sp. A 7, *Ponana* sp. B 2, *Prairiana* sp. A 5, *Prairiana* sp. B 4, *Oncometopia* sp. 1, *Diestostemma* sp. 1, *Draeculacephala mollipes* 3, *Cicadella sanguinolenta* 2, *Hortensia similis* 1, *Tylozygus fasciatus* 1, *Cicadellinae* sp. A 1, *Cicadellinae* sp. B 2, *Cicadellinae* sp.



Figs. 1—9. Spermatogonical metaphase plates in 9 species. — Figs. 1—6 BAB; Figs. 7—9 B.

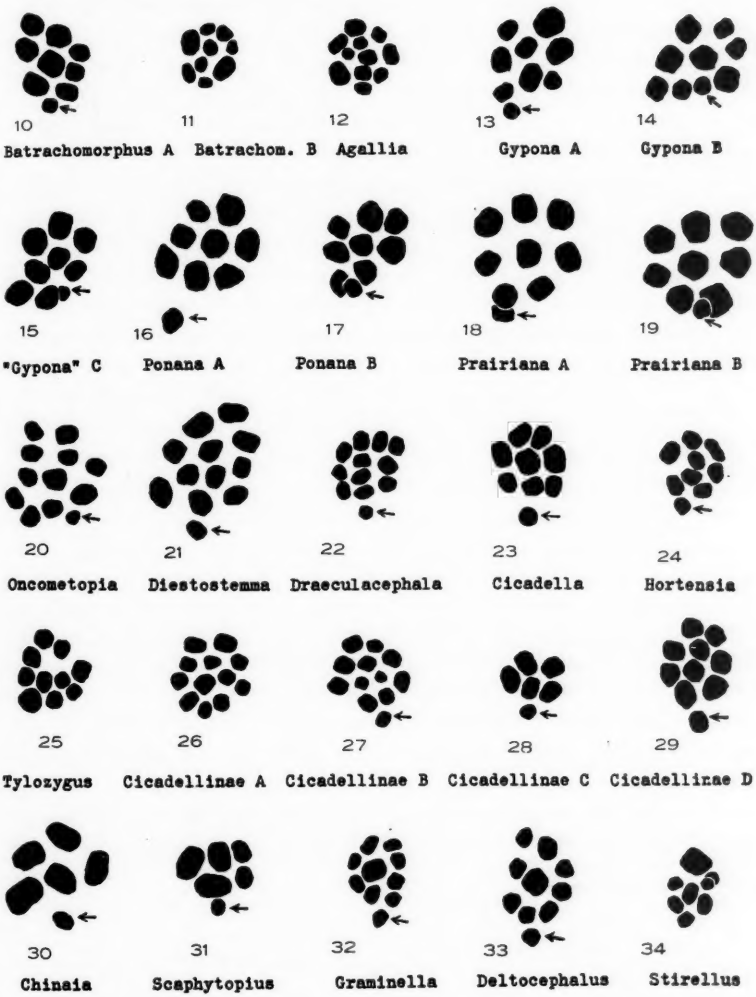
C 1, *Cicadellinae* sp. D 1, *Chinaia caprella* 1, *Scaphytopius dilatus* 5, *Graminella striatella* 1, *Deltocephalus flavicosta* 3, *Stirellus bicolor* 1.

All the individuals were males, the study being for practical reasons confined to this sex. About 80 % of the collecting was done at light traps kindly provided for the purpose by the personnel of the Barro Colorado Island Biological Station.

Only sections were made. The material was fixed in Bouin-Allen-Bauer (BAB), Benda (B) or Carnoy (C). The sections were cut at 15 μ. Only Feulgen's solution was used for staining. All the drawings were made with the aid of an Abbé camera lucida at table level, with the optics objective 100 $\times$, ocular 15 $\times$. The scale drawings so obtained represented a linear magnification of 4300:1 and after printing 2700:1.

CHROMOSOME SETS OF THE SPECIES INVESTIGATED

Spermatogonial metaphases were obtained from 9 species. Most of these represent a relatively early gonial cell generation and consequently show the size relations of the chromosomes with considerable accuracy. In the leafhoppers, late gonial cell generation metaphase chromosomes are usually roundish and small (Fig. 7). Although the tendency of the chromosomes to change size and shape during gonial



Figs. 10—34. First maturation division metaphase plates in spermatocytes in 23 species. Note: Figs. 11 and 34 show second division plates. The supposed X chromosome is indicated by an arrow. — Figs. 10, 18, 23, 27, 28, 30 BAB; Figs. 11—16, 19, 21, 22, 24, 29, 31, 32 B; Figs. 17, 20, 25, 26, 33, 34 C.

TABLE 1. *List of chromosome numbers.*

	2 n	I M	II M	sex chr	univ I A
Cicadellidae					
Iassides					
1. *Iassinae*					
Batrachomorphus (*Stragania*) n. sp. A Lv. in litt.	—	9	9, 8	XO	—
B. (S.) n. sp. B Lv. in litt.	(23)	(9)	— —	—	—
2. *Agalliinae*					
Agallia modesta Osb. & Ball	—	11	— —	(XO)	(+)
Cicadellides					
3. *Gyponinae*					
Gypona sp. A	—	9	9, 8	XO	—
Gypona sp. B	—	9	9, 8	XO	—
"Gypona" sp. C	17	9	9, 8	XO	+
Ponana sp. A	17	9	9, 8	XO	+
Ponana sp. B	—	9	9, 8	XO	+
Prairiana sp. A	17	9	9, 8	XO	+
Prairiana sp. B	17	9	9, 8	XO	+
4. *Cicadellinae*					
Oncometopia sp.	—	12	— —	XO	(+)
Diestostemma sp.	23	12	12, 11	XO	+
Draeculacephala mollipes Say coll.[1]	23	12	12, 11	XO	+
Cicadella sanguinolenta Coq. sensu Fowler	—	9	— —	XO	—
Hortensia similis Walk.	—	9	— —	(XO)	—
Tylozygus fasciatus (Walk.)	—	(10)	— —	—	—
Cicadellinae sp. A	—	12	12, 11	XO	+
Cicadellinae sp. B	23	12	12, 11	XO	+
Cicadellinae sp. C	11	6	6, 5	XO	+
Cicadellinae sp. D	—	10	— —	—	—
5. *Neocoelidiinae*					
Chinaia caprella Kramer	11	6	6, 5	XO	—
6. *Deltocephalinae*					
Scaphytopius dilatus Del.	—	6	6, 5	XO	+
Graminella striatella Lv.	—	(9)	— —	—	—
Deltocephalus flavicosta Stål	—	10	10, 9	XO	+
Stirellus bicolor V. Dz.	—	—	(8, 7)	(XO)	—

[1] *Draeculacephala mollipes* has been investigated by Boring (1907) under the generic name *Diedrocephala.*

development interferes with any effort to compare them in two given species, a comparison is possible when the ratio of thickness to length is about the same in the two species. This is true of *Cicadellinae* sp. C and *Chinaia caprella* (Figs. 8 and 9). The chromosomes of *Chinaia* are distinctly larger than those of the other species.

A similar difference in the size of the chromosomes exists between the above-mentioned species at the first metaphase stage (Figs. 28, 30). *Cicadellinae* sp. C and *Chinaia* belong to separate subfamilies, the latter representing the small subfamily *Neocoelidiinae* endemic to the New World. In all the Cicadelline species dealt with here, the average size of the chromosome is smaller than in *Chinaia* (Figs. 20—29 and 30). However, at least in *Diestostemma* sp. (Fig. 21) the total mass of the chromosomes is about the same as in *C. caprella*. *Diestostemma*, together with *Oncometopia*, belongs to the tribe *Proconiini*. All the other *Cicadellinæ* here investigated belong to the tribe *Cicadellini*.

The *Cicadellinae* and the *Neocoelidiinae* belong to the subfamily group *Cicadellides*, and the same is true of two other subfamilies included in the present study, the *Gyponinae* and the *Deltocephalinae*. The average size of the chromosomes is distinctly larger in the *Gyponinae* than in the *Cicadellinae* (Figs. 13—19 and 20—29). The subfamily *Gyponinae* seems to be quite uniform cytologically, with 8 bivalents and a univalent X chromosome in all the species investigated. The genus *Gypona* is characterized by having four autosomes larger than the others. The sex chromosome is never very large in the *Gyponinae*.

In the *Deltocephalinae*, *Scaphytopius dilatus* has 5 bivalents and a univalent sex chromosome (Fig. 31). The chromosomes are of about the same size as or a little larger than in *Cicadellinae* sp. C. The chromosomes of *Deltocephalus flavicosta* from Panama are almost identical with those of *D. pulicaris* from Finland (cf. Fig. 33 and Fig. 266 in HALKKA 1959).

Within the tribe *Cicadellini*, at least, the average size of the chromosomes seems to be inversely correlated with their number. As far as the observations go, the sex determination is of the XO type throughout.

In Table 1, the following abbreviations and symbols have been used:

In the univ I A column: +=negative heterokinesis of the X chromosome has been observed (succession).

In all the columns: —=no observations, parentheses ( )=because of limitations of material or for other reasons the report is only tentative.

DISCUSSION

The taxonomic relationships within the subfamily group *Cicadellides* of the Neotropical *Cicadellidae* have recently been discussed by LINNAVUORI (1959). He separates 12 subfamilies from the region. Of the four subfamilies represented in the present study, the *Neocoelidiinae* and the *Gyponinae* are Nearctic and Neotropical, while the *Cicadellinae* and the *Deltocephalinae* are cosmopolitan. The *Cicadellinae* is the largest subfamily, with more than 1000 species from the region, the *Gyponinae* and the *Deltocephalinae* (both about 500 species) being the next largest. The *Neocoelidiinae* is a small subfamily.

This discussion will deal exclusively with the four subfamilies mentioned above. Prior to the present work, 68 species belonging to the subfamily group *Cicadellides* had been studied cytologically. In the earlier works 6 subfamilies (*Ulopinae, Eupelicinae, Xerophloeinae, Aphrodinae, Cicadellinae* and *Deltocephalinae*) were included. In the present work, 22 species from the *Cicadellides* have been investigated (Table 1), which brings the total for the subfamily group up to 90.

According to LINNAVUORI (op. cit.), the systematic position of the *Gyponinae* has aroused some controversy among taxonomists. A relationship with forms like *Xerophloea* has been claimed by earlier workers, but LINNAVUORI suggests some possible kinship with the *Aphrodinae*. Cytologically, the *Gyponinae*, with their 9 large chromosomes, come close to the Aphrodine species *Strongylocephalus agrestis* (which has 10 chromosomes; HALKKA, 1959). The *Gyponinae* afford a further case of the presence of a foliaceous anterior margin of the head and unusually large chromosomes. The list of subfamilies showing this correlation now comprises the *Ulopinae, Eupelicinae, Xerophloeinae* (=*Ledrinae* in HALKKA, 1960), *Aphrodinae* and *Gyponinae*. Thus, the cytological evidence would suggest placing the *Gyponinae* somewhere not far from the base of the family tree for the *Cicadellides* (cf. HALKKA, 1959).

In the *Cicadellinae*, the existence of a number as low as 6 raises some questions. The number happens to be exactly half the modal number of the subfamily (Fig. 35). Most probably the numbers 12 and 6 are related to each other by the (transverse) fusion-fragmentation principle, which implies that the case would parallel the *Eurhadina* case (subfamily group *Typhlocybides*) observed by HALKKA (1959).

LINNAVUORI (1959) suggests for the *Neocoelidiinae* a very close relationship with the *Deltocephalinae*. On the basis of the chromosomes

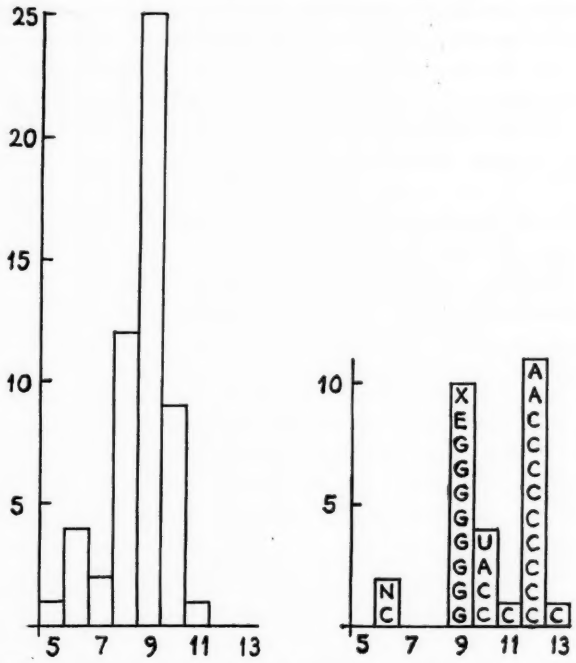


Fig. 35. Histograms indicating haploid chromosome numbers in 8 subfamilies belonging to the subfamily group *Cicadellides*. Figures on the ordinates represent numbers of species. — Blank=*Deltocephalinae*, A=*Aphrodinae*, C=*Cicadellinae*, E=*Eupelicinae*, G=*Gyponinae*, N=*Neocoelidiinae*, U=*Ulopinae*, X=*Xerophloeinae*.

of a single species nothing can be said about this, except perhaps that no feature in the cytology of *Chinaia* is incompatible with his assumption.

In the family *Cicadellidae*, there exist at least two levels of polyteny, that occurring in *Xerophloea viridis* (*Xerophloeinae*) being four times that observed in *Graphocephala coccinea* (*Cicadellinae*) (HALKKA, 1960). Thus, it is very probable that a third level, intermediate between the *Xerophloea* and *Graphocephala* levels, will also be met with. In the material of the present work, the chromosomes of certain Gyponine species do not fall far short of the *Xerophloea* chromosomes in size, while those of *Draeculacephala* are about equal to the *Graphocephala* chromosomes.

Although it is, of course, somewhat pointless to speak in terms of

TABLE 2. *Frequency of ring bivalents in the Cicadellidae.*

	I					II	III	IV
	0	1	2	3	4			
1. *Iassides*								
Oncopsis alni	8	32	29	28	3	9 + XY	2	77 + 23 = 100
Oncopsis tristis	59	27	13	1	0	9 + XY	2	82 + 40 = 122
Idiocerus populi	11	54	32	3	0	11 + XO	2	35 + 28 = 63
2. *Typhlocybides*								
Notus flavipennis	2	85	13	0	0	8 + XO	1	33
Empoasca apicalis	68	32	0	0	0	8 + XO	1	101
3. *Cicadellides*								
"Gypona" sp. C	85	15	0	0	0	8 + XO	1	100
Cicadellinae sp. B	100	0	0	0	0	11 + XO	1	100
Cicadellinae sp. C	60	40	0	0	0	5 + XO	1	20
Cicadella viridis	100	0	0	0	0	9 + XO	3	60
Athysanus argentarius ..	1	98	0	0	0	8 + XO	2	20 + 30 = 50
Macustus grisescens	99	1	0	0	0	7 + XY	3	100
Scaphytopius dilatus	100	0	0	0	0	5 + XO	2	100 + 100

Part of the results based on Finnish material. — I=percentage of nuclei with 0, 1, 2, 3 and 4 ring bivalents, II=autosome bivalents and sex chromosomes in the species investigated, III=number of specimens investigated, IV=number of nuclei investigated.

"polyteny levels" in the Panamanian leafhoppers without having measured any actual DNA values, the differences in the sizes of identically fixed and stained chromosomes from the different subfamilies are great enough to warrant some remarks on this point. Apparently the *Cicadellides*, as well as the other subfamily groups of the *Cicadellidae*, is a group in which changes in polyteny on the one hand and transverse fusions and fragmentations on the other have played important roles in the evolution of subfamilies and tribes.

In the Heteropteran family *Pentatomidae*, SCHRADER and HUGHES-SCHRADER (1956) have found differential polyteny associated with a doubling of chromosome number in the genus *Thyanta*. In this genus there appears to be only one pairing block in each bivalent and all the bivalents are monochiasmate. Although the chiasma frequency is low in the *Auchenorrhyncha*, ring bivalents are not very uncommon and are met with in all subfamily groups of the *Cicadellidae* (Table 2). In *Idiocerus populi* (*Iassides*, *Idiocerinae*), multivalents have been found in tetraploid spermatocytes (HALKKA, unpublished observations). Probably in

many members of the *Cicadellidae* a doubling of the number of chromosomes, whether accompanied by a change in polyteny or not, would result in multivalent formation. This, together with the impaired sex chromosome conditions (the sex chromosomes of the *Auchenorrhyncha* behave differently from those of the *Pentatomidae*) would render it practically impossible for the doubled number to become established permanently.

That differences in polyteny are probably never accompanied by a change in chromosome number does not mean that they are without evolutionary significance in the *Auchenorrhyncha.* Provided that readily observable size differences such as exist between *Cicadellinae* sp. C and *Chinaia*, really reflect a difference in the degree of polyteny, it seems that most changes in polyteny are of relatively ancient origin. Within subfamilies and tribes the size of the chromosomes does not vary very much, but in different subfamilies and tribes the average size of a chromosome set is often characteristically dissimilar.

Superimposed on these assumed changes in degrees of polyteny are numerous transverse fragmentations and fusions. They have led to minor (and some major) differences in chromosome numbers between the genera, and occasionally within a genus too. Examples are provided by HALKKA (1959), who believes that the main evolutionary trend has been towards smaller chromosome numbers. Of course, the author's opinions on chromosomal evolution within the *Cicadellidae* and the *Auchenorrhyncha* in general are entirely speculative, but for the present the hypothesis seems to explain a larger number of facts than any alternative.

The low frequency of chiasmata in the *Cicadellidae* warrants some remarks about the possible role of chiasma interference. In leafhoppers often only the largest bivalent has the form of a ring at late diakinesis. Examples: *Eupelix cuspidata, Graphocraerus ventralis, Athysanus argentarius, Coryphaelus gyllenhali* (HALKKA, 1959), *Xerophloea viridis* (HALKKA, 1960). These published observations are supported by a number of unpublished ones.

The chiasma frequency seems to be particularly low in the *Cicadellides* group of subfamilies (Table 2). All the five species mentioned above belong to this subfamily group. It appears that the chromosomes must attain a certain minimum length for two chiasmata to be formed in a bivalent. This minimum length is generally reached only when (probably through fusions or translocations) one of the chromosomes is distinctly larger than the rest.

In the light of the observations quoted above it seems safe to assume that a relatively strong chiasma interference, extending through the whole length of an average Cicadellid chromosome, is a characteristic feature of leafhopper meiosis. The centromere being diffuse, and centromere-associated heterochromatin thus absent, chiasma interference is able to exert its influence without restriction in both directions. Chiasma interference may also partially account for the possible terminal localization of chiasmata in certain leafhoppers (cf. HALKKA 1960, p. 258).

The diffuseness of the Auchenorrhynchan centromere, here taken for granted, has recently been challenged by SESHACHAR, RAO and DASS (1959). The present author regards the evidence afforded by the X-ray experiments of SESHACHAR et al. (op. cit.) as inconclusive. First, the choice of a species with a relatively large number of chromosomes (n=13) makes interpretation of results difficult. Secondly, the lagging fragments observed in their studies may well result from induced stickiness of the chromosomes. In the Auchenorrhyncha studied by the present author all the morphological and cell mechanical criteria tend to substantiate the existence of a diffuse centromere.

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Dr. M. MOYNIHAN, as well as all the other personnel at the Barro Colorado Island Biological Station most generously provided me with good collecting facilities. My visit to Barro Colorado was arranged through the Smithsonian Institution, which very helpfully cleared all the entrance formalities.

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The species investigated have been determined by Dr. R. LINNAVUORI. I express my deep gratitude to him for this. The English of my manuscript was checked by Mrs. JEAN-MARGARET PERTTUNEN, M.A., to whom my sincere thanks are due.

SUMMARY

To the list of cytologically investigated *Auchenorrhyncha*, 25 Panamanian species belonging to the family *Cicadellidae* are added. The relative roles of differential polyteny and transverse fusions and frag-

mentations in the chromosomal evolution of the *Cicadellidae* are briefly discussed. Evidence for the existence of chiasma interference is provided.

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CHROMATIN EXTRUSION AND CYTOMIXIS IN POLLEN MOTHER CELLS OF HORDEUM

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CHROMATIN extrusion and cytomixis or the transfer of nuclear material from one cell to an adjacent cell has been reported in many plant genera for a number of years (SARVELLA, 1958).

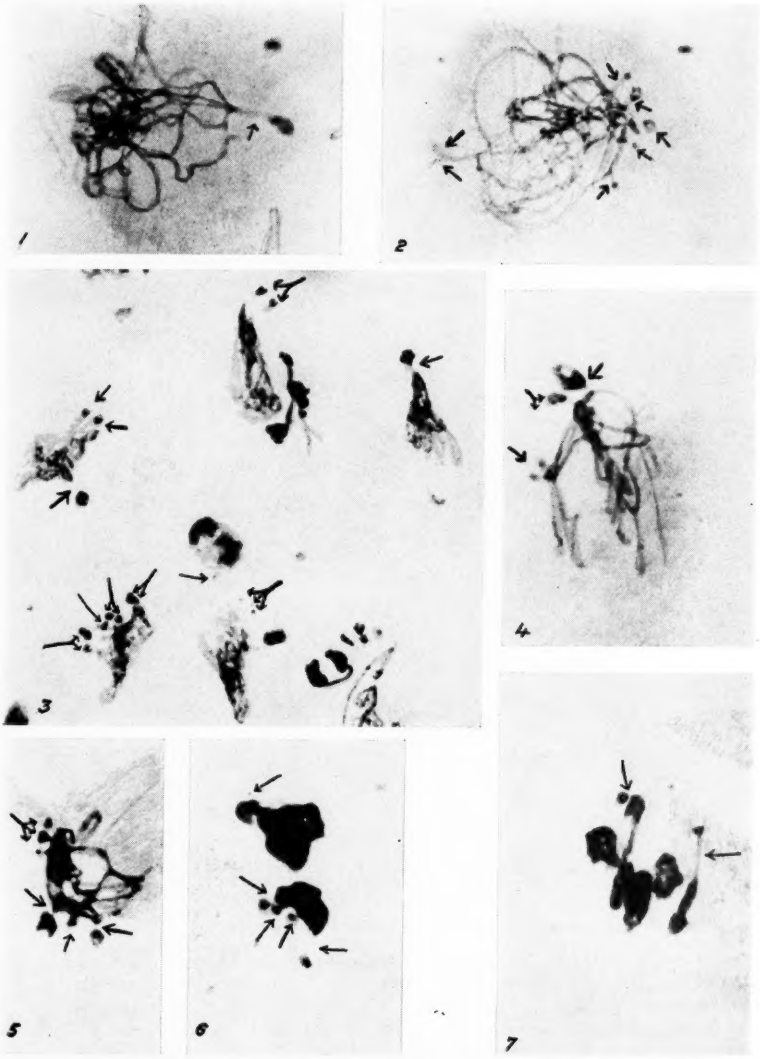
During cytological analysis of a number of barley mutants induced by X-rays and neutrons many pollen mother cells were found with a clear evidence of chromatin extrusion and transfer of chromatin from one cell to another. In the present paper this cytological evidence, along with a bibliography on chromatin extrusion and cytomixis is presented.

Spikes of barley (*Hordeum vulgare*) containing pollen mother cells (PMC) in meiosis were fixed, stained by the Feulgen method after hydrolysis and squashed. The material used for the cytological studies was partially and completely sterile mutants induced in erectoides-14 and erectoides-23, exhibiting heterozygosity for one or more translocations, homozygosity for asynapsis, failure of cytokinesis, etc.

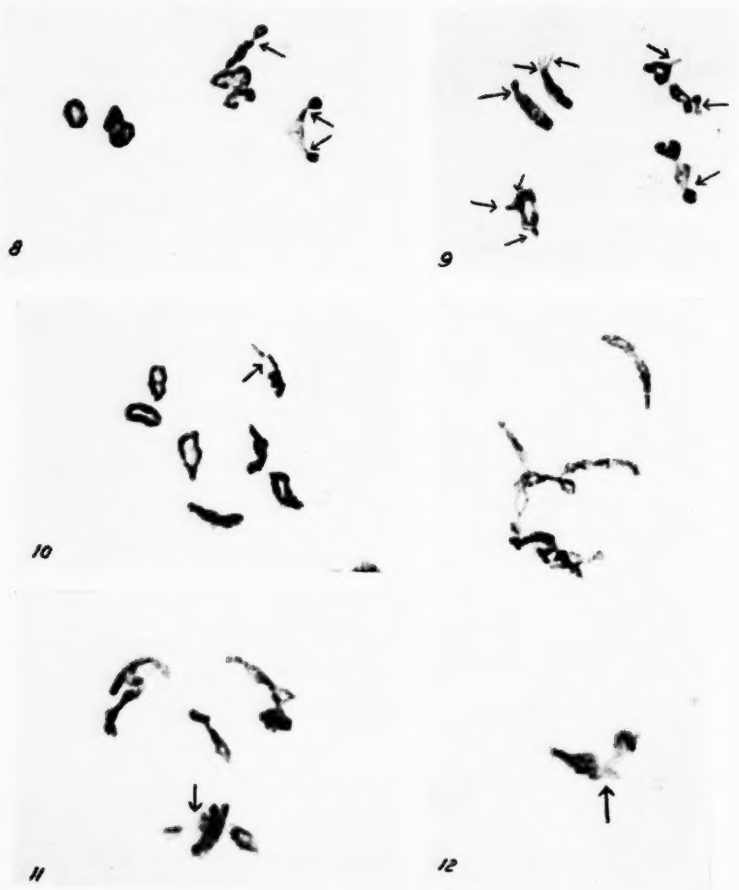
OBSERVATIONS

Evidence of chromatin extrusion and cytomixis was clearly seen in several PMC in the various barley mutants studied. The proportion of PMC exhibiting the phenomena varied in different lines. In some sterile mutants as much as 40 per cent of the PMC showed evidence of chromatin extrusion and cytomixis (Fig. 3).

The earliest signs of chromatin extrusion were seen at early pachytene. At pachytene the chromatin material could be seen as protruding out of the main chromatin mass at one or more places (Figs. 1 to 5). Protrusions ranging in number from one (Fig. 1) to eight (Fig. 3) or more could be easily seen in the various PMC. Signs of chromatin extrusion occurring in the early stages persisted in the later ones and



Figs. 1 to 5. Photomicrographs of pollen mother cells at pachytene and early prophase I with evidence of chromatin extrusion (shown with darts). The extruded chromatin bodies may vary in number from one (Fig. 1) to several (Figs. 2 to 5). — Figs. 6 and 7. Photomicrographs of pollen mother cells at metaphase I with evidence of chromatin extrusion (shown with darts). Chromatin bodies can be seen as protrusions budding out of the bivalents.



Figs. 8 and 9. Evidence for chromatin extrusion at metaphase I (Text same as for Figs. 6 and 7). A bivalent may show one (Fig. 7), two (Fig. 8) or several (Fig. 9) chromatin protrusions. — Figs. 10 to 12. Photomicrographs of pollen mother cells at diakinesis and diplotene with evidence of chromatin extrusion (shown with darts). Chromatin pieces have been lost from these cells at earlier stages resulting in the formation of "torn bivalents".

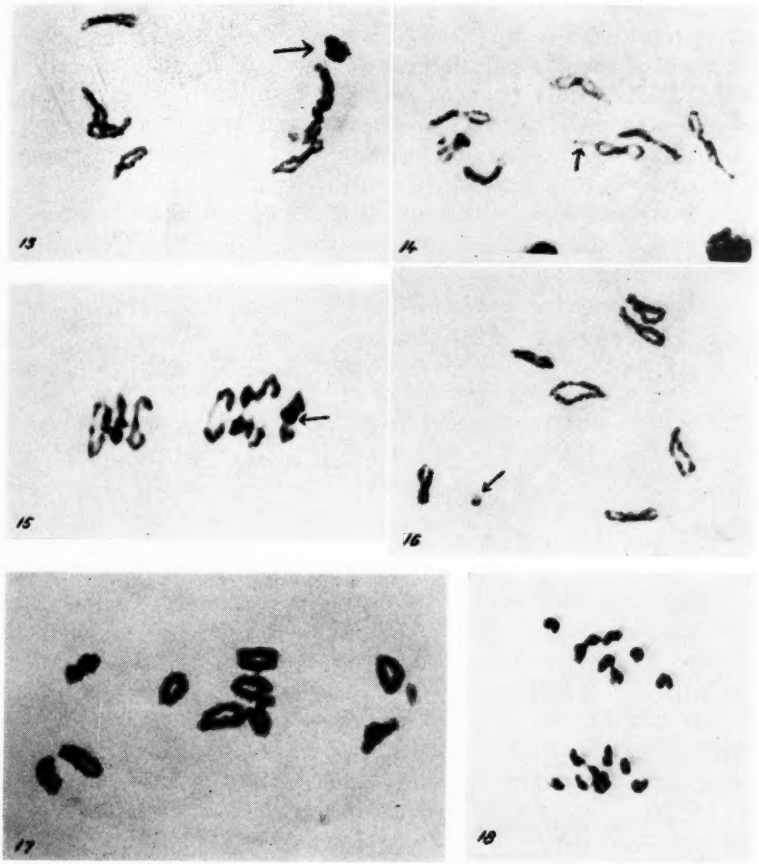


Fig. 13. Photomicrograph of a pollen mother cell at early diakinesis showing an extra mass of chromatin received by extrusion from an adjacent cell. — Figs. 14 to 16. Photomicrographs of pollen mother cells at diplotene, metaphase I and diakinesis respectively, showing an extra fragment (indicated by dart) that may have originated through chromatin extrusion and cytomixis. The extra fragment in Fig. 15 seems to have centromeric activity. — Figs. 17 and 18. Photomicrographs of pollen mother cells at diakinesis and anaphase I showing the presence of extra bivalents that probably have arisen through cytomixis.

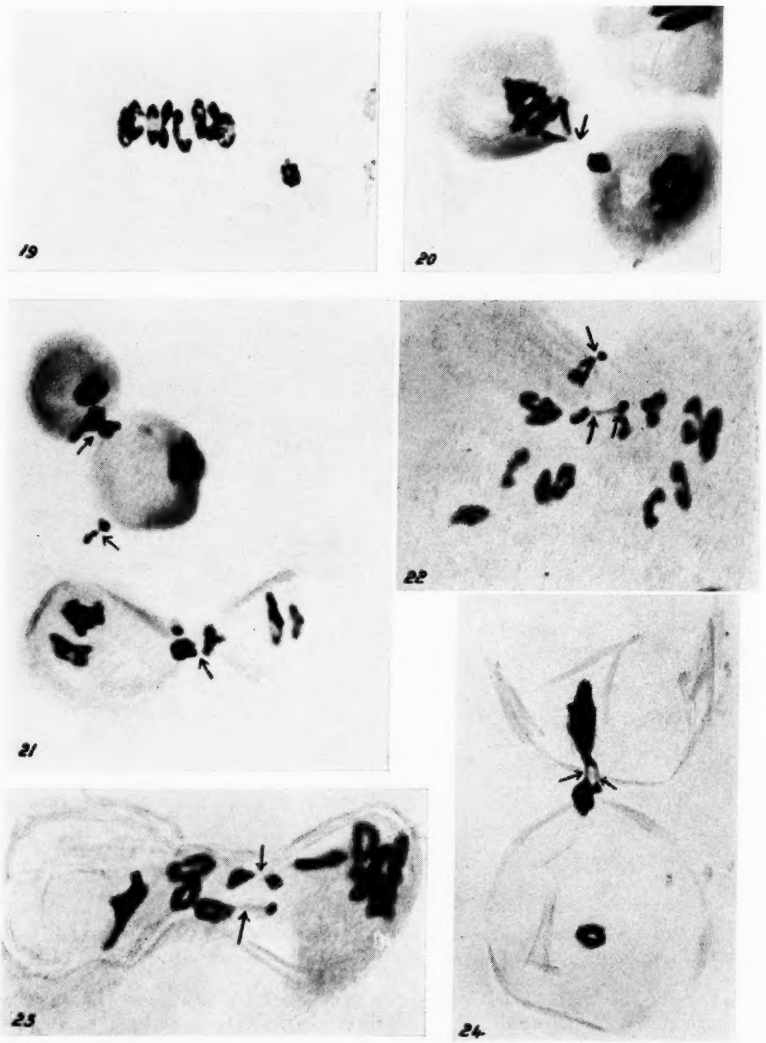


Fig. 19. Photomicrograph of a pollen mother cell at metaphase I showing the presence of extra bivalents that probably have arisen through cytomixis. — Figs. 20 to 24. Photomicrographs of pairs of pollen mother cells showing the chromatin links between PMC. Here cytomixis is incomplete. Chromatin bodies budding out of bivalents (remnants of chromatin extrusion) may be seen in all the cells.

could easily be recognised. For instance, in diakinesis and metaphase I, one or more of the protrusions may be visible as Feulgen-positive bodies budding out of a bivalent (Figs. 6 to 9). However, more commonly they get detached and are seen as one or more Feulgen-positive bodies in the cytoplasm of the same PMC (Fig. 7) or that of an adjacent PMC (Fig. 13). The former would be an example of chromatin extrusion and the latter of cytomixis. The number of bivalents exhibiting signs of chromatin extrusion may differ in different cells, ranging from one to seven (Fig. 9). The PMC losing pieces of chromatin may be expected to be non-functional and may contribute to the lethality of the gametes.

If chromatin pieces are lost or transferred by extrusion and cytomixis, one should observe PMC with abnormal chromosome complements. This is in fact what is observed. Many PMC, when cytologically examined, were found to be lacking in parts of chromosomes or had some extra pieces of chromatin material. Cells which have lost pieces of chromatin at an early stage like pachytene should contain 'torn bivalents' at later stages. Examples of PMC with torn bivalents can be seen in Figs. 10 to 12. In Fig. 13, a PMC at early diakinesis is seen as having a mass of extra chromatin which must have been received from an adjacent PMC.

The amount of chromatin extruded from any PMC varies greatly. It may vary from a small piece to the entire chromosome complement of a nucleus, in different PMC in the same anther. More often, just a piece or one bivalent is extruded. If the piece extruded happens to have centromeric activity, it may be accepted by the spindle apparatus of the recipient cell. This may eventually give rise to gametes with an additional centric fragment. If such a gamete is functional a new source of additional genetic material is available in the population. Cytological evidence of PMC with an extra fragment was obtained in five cells (Figs. 14 to 16) out of several hundred PMC showing chromatin extrusion and cytomixis. Of these, in only one PMC did the fragment show centromeric activity (Fig. 15).

Pollen mother cells with extra bivalents originate more frequently through chromatin extrusion and cytomixis than cells in which only an extra fragment is added. PMC with 10 bivalents at diakinesis, 8 bivalents at anaphase I and 9 bivalents at metaphase I can be seen in Figs. 17, 18 and 19 respectively. These PMC were found in the same anthers which also showed PMC with characteristic signs of chromatin extrusion and cytomixis. Thus it is highly probable that the chromosome increase has come about through cytomixis, completed at a previous

stage, although no signs of cytomixis persisted at this stage, except an increase in the number of chromosomes.

The extra chromosomes added cytomictically may all be included in the spindle apparatus of the recipient cell (Fig. 18) or only some of them may be included (Fig. 19). In Fig. 19, can be seen a PMC at metaphase I with nine bivalents. One of the extra bivalents is lying alone and outside the spindle apparatus of the normal complement whereas the other is included in it.

Many of the PMC showing chromatin extrusion and cytomixis were such that the transfer of chromatin was incomplete (Figs. 20 to 24) and one (Fig. 20) or more (Figs. 21, 23 and 24) chromatin links between PMC could be seen. Usually one of the PMC has the full chromosome complement (recipient) and the other (donor) shows one or more of the bivalents being extruded (Figs. 20, 21, 23 and 24). However, this is not always the case, *e.g.*, in Fig. 22, one bivalent from each of the two PMC is showing signs of cytomixis.

DISCUSSION

It is important to clarify the relationship and the distinction between chromatin extrusion and cytomixis. The two terms have been used by different authors to designate different phenomena. The phenomenon of transfer of chromatin material from one cell to another may be termed as cytomixis. Chromatin extrusion occurs at an early stage in meiotic division like pachytene persisting in the bivalents in later stages and may be seen as protrusions from the paired chromosomes. It is inferred from the presence of such bivalents in PMC showing evidence of cytomixis in the form of links between adjacent cells, that extrusion of chromatin may precede cytomixis in some cases. It may be admitted, however, that PMC with extra bivalents which must have arisen from cytomixis, but with no signs of chromatin extrusion have also been observed.

Chromatin extrusion and cytomixis have been reported in many plant genera for a long time (Table 1). Variations in the direction of chromatin movement have been observed in different organisms. In *Oenothera gigas* (GATES, 1911) cytomixis occurred at the same time in all the PMC of an anther and always in the same direction in all the cells. In *Lilium* (WEST and LECHMERE, 1915) the direction of cytomixis was not always the same in all the PMC. KATTERMANN (1933) also ob-

TABLE 1. *Instances of chromatin extrusion and cytomixis in plants.*

Experimental material	Authors
Crocus vernus	KÖRNICKE, M. 1901
Lathyrus odoratus	GREGORY, R. P. 1905
Galtonia canadensis	DIGBY, L. 1909
Drosera longifolia×*D. rotundifolia*	ROSENBERG, O. 1909 a and b
Oenothera gigas	GATES, R. R. 1911
Wheat and hybrids	NAKAO, M. 1911
Crepis virens	DIGBY, L. 1914
Vicia faba	FRASER, A. C. I. 1914
Lilium candidum	WEST, C. and LECHMERE, A. E. 1915
Lactuca	GATES, R. R. and REES, E. M. 1921
Papaver hybrids	YASUI, K. 1922
Iris japonica	SINOTO, M. 1922
Nolana	CAMPIN, M. G. 1925
Lathraea	GATES, R. R. and LATTER, J. 1927
Nicotiana tabacum	RUTTLE, M. L. 1928
Iris Kaempferi	INARIYAMA, S. 1929
Zea mays	MCCLINTOCK, B. 1929
Corylus and *Alnus*	WOODWORTH, R. H. 1929
Aegilops×wheat	PERCIVAL, J. 1930
Saxifraga potternensis	WHYTE, R. O. 1930
Thespesia populnea	YOUNGMAN, W. 1931
Wheat	POWERS, L. 1932
Antennaria	STEBBINS, G. L. 1932
Wheat×rye	KATTERMANN, G. 1933
Triticum aegilopoides×*Aegilops squarrosa*	KIHARA, H. and LILIENFELD, F. 1934
Festuca×*Lolium*	PETO, F. H. 1934
Hemp	BRESLAVETZ, L. P. 1935
Phacellanthus tubiflorus	MATSUURA, H. 1935
Oryza sativa	NANDI, H. K. 1937
Triticum	YAMASHITA, K. 1937
Gladiolus	MENSINKAI, S. W. 1939
Secale cereale	MÜNTZING, A. and PRAKKEN, R. 1941
Sagittaria natans	VAARAMA, A. 1941
Rumex acetosella	LÖVE, A. 1943
Alopecurus myosuroides	JOHNSSON, H. 1944
Primula and *Cyclamen*	NYBOM, N. 1946
8 species of plants	SPARROW, A. H. and HAMMOND, M. R. 1947
Review on cytomixis	MAHESHWARI, P. 1950
Aloë sp.	MENDES, E. J. and RIJO, L. 1951
Review on cytomixis	TISCHLER, G. 1951
17 species of Angiosperms	COOPER, D. C. 1952
Secale cereale×*Agropyron intermedium*	GAUL, H. 1954
Salvia	LINNERT, G. 1955
4n and 6n *Gossypium* amphiploids, *Nicotiana tabacum* and *Solanum quitöense*	SARVELLA, P. 1958
Gasteria trigona	BOPP-HASSENKAMP, G. 1959
5 species of Angiosperms	TAKATS, S. T. 1959
Hordeum mutants	KAMRA, O. P. (present study)

served a random movement and contents of PMC could be seen moving in two or more directions at the same time. In *Phacellanthus tubiflorus* (MATSUURA, 1935) although some regularity in the direction of extrusion was noticed, cases where the direction of extrusion was opposite to that of the migration of nuclear contents were also seen. In the present investigation the direction of chromatin extrusion and cytomixis seems to be non-uniform. The nucleolus may also be involved in cytomixis (YOUNGMAN, 1931) or it may not be affected (GATES, 1911). The nucleolar condition could not be ascertained in the present study due to the staining procedure employed.

In many studies, cytomixis has been reported to occur at early stages of meiosis (KIHARA and LILIENFELD, 1934; MATSUURA, 1935) although it has been observed at metaphase I and anaphase I (SARVELLA, 1958) and as late as telophase II (STEBBINS, 1932). On the other hand, chromatin extrusion has not been observed later than prophase I (MATSUURA, 1935; TAKATS, 1959). The absence of extrusion at later stages have led some investigators to postulate that the affected cells either undergo recovery or simply degenerate. In the present investigation chromatin extrusion and cytomixis have been seen in early prophase, metaphase I and anaphase I.

Cytomixis can give rise to PMC with increased chromosome numbers. In *Corylus* (WOODWORTH, 1929) abnormal chromosome numbers in metaphase II were considered to be due to cytomictical migration or chromosome fusion. Similar observations were made in *Aegilops ovata*$\times$ *Triticum monococcum* (PERCIVAL, 1930) and *Gossypium hirsutum*$\times$*G. barbadense* (SARVELLA, 1958). Cells with decreased chromosome numbers can also arise cytomictically (KIHARA and LILIENFELD, 1934; YAMASHITA, 1937; SARVELLA, 1958). In the present study, deviations in chromosome number, both positive and negative, were found in the PMC of barley mutants.

There is speculation that binucleate PMC originate through cytomixis in *Nicotiana tabacum* (RUTTLE, 1928), *Phacellanthus tubiflorus* (MATSUURA, 1935), *Oryza sativa* (NANDI, 1937) and 4n and 6n *Gossypium* amphiploids (SARVELLA, 1958).

The occurrence of cytomixis has been doubted in many cases because of the unsuitability of the material under investigation. It has been shown that the proportion of PMC showing cytomixis can be varied depending upon the extent of wounding of the anthers, fixative used and the method employed in the preparation of slides (TAKATS, 1959). This may be true to some extent. Nevertheless, there is no doubt that

cytomixis does occur during meiosis though infrequently. Many investigations carefully executed confirm its occurrence (MATSUURA, 1935; SARVELLA, 1958). The material used in the present study, *viz.* barley, is ideally suited for detailed analysis of chromosomes, this being very difficult in a number of the earlier experiments.

It has been suggested that typical configurations showing cytomixis can arise due to defective squashing (LINNERT, 1955). In the author's opinion, this is not entirely valid. No amount of defective squashing or application of pressure could produce such small protrusions so close to one another as evident in Fig. 2, or cause the formation of torn bivalents (Figs. 10, 11 and 12) and produce gametes with extra fragments (Figs. 14, 15 and 16) or increase the number of bivalents in the PMC (Figs. 17—19), specially at anaphase. On the other hand, cells torn by squashing may lead to the loss of one or more bivalents, and for this reason, cells with less than seven bivalents have not been included as evidence of cytomixis.

The nature and function of chromatin extrusion have been subjects for speculation by many investigators. Among others, the following hypotheses are worthy of note: (1) chromatin extrusion causes abnormal divisions of PMC (GREGORY, 1905); (2) extruded bodies are waste materials (WEST and LECHMERE, 1915); (3) extruded bodies either serve as a site for protein synthesis or may be converted into ribose nucleic acid in the cytoplasm of the sporocyte (SPARROW and HAMMOND, 1947); and (4) extruded bodies are utilized by the nucleus at the onset of meiosis (DARLINGTON and LACOUR, 1946; COOPER, 1952). COOPER (1952) thought that the chromatin bodies were extruded from the tapetum and taken up by the cytoplasm of the PMC. Thus tapetum was suspected of providing some deoxyribose nucleic acid (DNA) which triggered the duplication of DNA in the PMC. This has not been verified in plant material. TAKATS (1959), with the aid of isotopic labelling, has shown that the extruded bodies are not derived from tapetum DNA but arise from the PMC themselves. However, a similar event probably occurs during spermatogenesis in a number of insects. SCHRADER (1951), SCHRADER and LEUCHTENBERGER (1952) and BONHAG (1955) have found that certain nuclei in the ovarian chamber regularly degenerate and presumably are converted into Feulgen-negative material which may be essential to the metabolism of the developing meiotic cells.

The importance of cytomixis is still obscure, with the probable exception of the formation of unreduced gametes in a very low frequency. However, cytomixis may also give rise to gametes with extra fragments

as indicated by the cytological evidence presented here. If these gametes are functional, there would be a resultant increase in the amount of genetic material.

SUMMARY

Chromatin extrusion and cytomixis were observed in the pollen mother cells (PMC) of several barley mutants. A detailed cytological analysis of cytomictic cells was possible owing to the favourable cytology of barley revealing the presence of extruded bodies budding out of bivalents at first meiotic division. The evidence presented for chromatin extrusion and cytomixis includes the presence of Feulgen-positive chromatin bodies, torn bivalents, additional chromosome fragments, cells with extra bivalents and chromatin links between adjacent PMC in different stages of meiosis.

The significance of cytomixis in meiosis is discussed and the possibility of gametes with extra fragments arising due to cytomixis is indicated.

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THE SELECTION OF *st* AND *cn* ALLELES ON DIFFERENT GENETICAL BACKGROUNDS IN *DROSOPHILA MELANOGASTER*

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I. INTRODUCTION

MOST mutant genes are at a disadvantage compared to wild type alleles, *i.e.* in competition with a wild type allele the mutant allele will decrease in frequency in a population. Very often this results in the elimination of the mutant allele from the population. The most common exceptions are mutant alleles which endow the heterozygote with higher fitness than both homozygotes, resulting in a frequency equilibrium in the population.

The rate of elimination as well as the rate of approach to a possible equilibrium depend primarily on the alleles concerned, but the rate of frequency change can often be modified considerably by substitutions in the general genetical background. Most of the modifications brought about by changes in the genetical background must be accepted at face value in the sense that it remains unknown in genetical as well as in physiological terms why the relative selective values of the alleles involved are changed.

The present paper is concerned with known substitutions in the background of such a kind that it should be possible on physiological grounds to make predictions as to the influence of the substitutions on the relative selective values of a pair of alleles. The argument is the following.

Consider three loci, *a*, *b*, and *c*, acting in such a way that a^+ controls the production of the precursor for b^+, which in turn supplies c^+ with its precursor. If an amorphic or hypomorphic mutant (MULLER, 1932) of *c* is at a disadvantage compared with c^+ in a population homozygous for a^+ and b^+, this disadvantage is presumably due to the decreased

amount of the c^+ product in c/c individuals, and possibly in c/c^+ as well. If, however, c competes with c^+ in a population which is homozygous for an amorphic allele of the a or the b locus (or both), the difference in selective value of c *versus* c^+ should be grossly diminished, as this locus is no longer supplied with any precursor. Changes in the relative selective values of c and c^+ may also be expected if loci concerned with biochemical steps prior to that of c^+ are made homozygous for hypomorphic or hypermorphic alleles, although in these cases it is less obvious which kind of change to expect.

The *vermilion* group of loci in *Drosophila melanogaster* constitutes an example of three loci acting in the same biochemical chain and leading to the production of brown eye pigment. For the present study we have chosen the *v*, *cn*, and *st* loci of this group. The sequence of physiological actions of these loci is $v^+ \rightarrow cn^+ \rightarrow st^+$. The v^+ allele controls the conversion of tryptophane into kynurenine, which is further converted by the cn^+ allele into hydroxykynurenine. The st^+ allele is essential for the formation of brown chromophore from hydroxykynurenine and the so-called w^+ substrate (NOLTE, 1959), but the exact nature of the action of the st^+ allele is still unknown. Flies homozygous for mutant alleles of these three loci have bright red eyes indicating a considerable decrease in the amount of brown eye pigment. Spectrophotometric measurements (NOLTE, 1954) have revealed that the amount of brown pigment in relation to red pigment in these mutants is decreased to about 5 % of that found in wild type flies.

Applying the above argument to this series of loci, the following more obvious predictions have been made:

1) If a mutant *st* is at a disadvantage compared with st^+ in a v^+/v^+, cn^+/cn^+ population, in the sense that *st* decreases in frequency, the selection against *st* should be suspended or at least should slow down rather than accelerate when the population is made homozygous for an amorphic mutant at the *cn* or the *v* locus.

2) If *cn* is at a disadvantage compared with cn^+ in a population homozygous for st^+ and v^+, the selection against *cn* should be suspended or at least should slow down rather than accelerate when v^+/v^+ is substituted with v/v.

These predictions have been tested by observing the frequency changes of the involved alleles in a number of artificial populations.

II. MATERIALS AND METHODS

Strains used. The two mutant alleles whose selectional behaviour has been investigated are 1) a mutant allele of the *cn* locus, here called cn^s to distinguish it from other *cn* alleles available in this laboratory, and 2) an *st* allele. The origin of these alleles are unknown, but both strains have been kept in culture for several years. None of the mutants are associated with major chromosomal aberrations. Three wild type strains have been employed, *viz.* 1) an *Oregon* strain; 2) a Danish wild type strain called *Holte* (from its place of collection), which by the time it was used in the experiments to be described below had been in the laboratory for only about six months; 3) *a-3* which is a wild type strain derived from a natural population near Madison, Wisconsin, USA. The *a-3* has been in culture for about 5 years and has gone through more than ten generations of brother-sister mating. Both the *Oregon* and the *a-3* strain are rather closely inbred, whereas the *Holte* strain most probably contained a large amount of genetic variation at the time it was used in this study. 4) Finally, a *vermilion* strain has been used. Its origin is unknown but it has been kept in this laboratory for several years.

The population boxes. All populations described below were kept in the Bennett plastic population boxes (BENNETT, 1956; FRYDENBERG, 1958). Each box (see Fig. 1) has on two of its sides four holes into which a standard *Drosophila* vial (25×100 mm) with 8 cm^3 of standard *Drosophila* medium can be inserted. One food vial was renewed every second day so that any vial stayed 16 days in the box at which time its contents were used up leaving only empty pupae and stray flies. Care was taken when changing the vial to prevent the escape of too many flies from the box, and, more important, to avoid possible contamination of the box by foreign flies. One can never be completely sure that no contamination has occurred, but it does seem to be a very rare event. Among the more than 100 different boxes kept continuously in this laboratory, several carry one or more recessive markers (say, *cn*, *bw*, *e*) so that a possible contamination would be followed in most cases by the occurrence of wild type flies in the box. These contamination-control boxes are treated exactly as the experimental boxes; actually the technical personnel changing the food vials do not know the difference between the two kinds of boxes. None of the control boxes has ever shown any sign of contamination.

All boxes are kept in a temperature room at 25° C ± 1°. A continuous

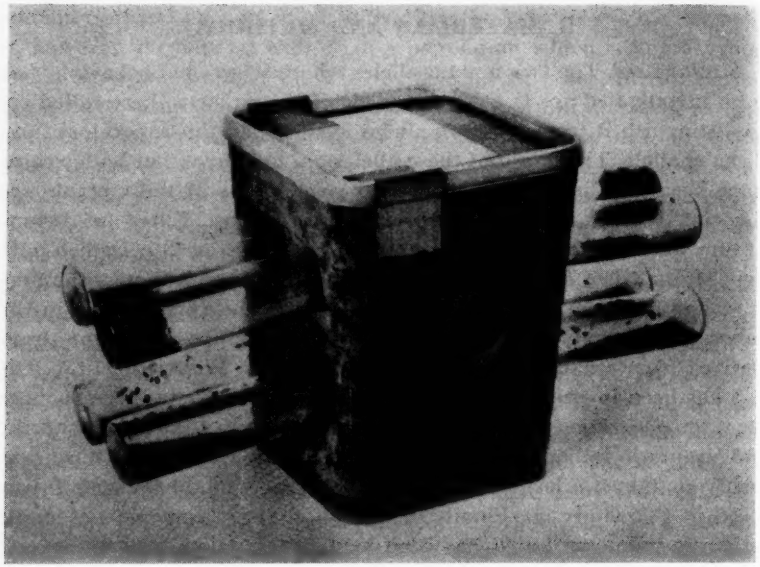

Fig. 1. The population cage model used in this study. Its dimensions are 10×10×14 cm.

temperature record is kept. It has happened two or three times during the experiment that the room temperature has deviated more than stated, but this has never been of more than a day's duration. A number of experiments with different strains of flies have shown that the average generation time in the Bennett boxes kept under our standard conditions is between 13 and 15 days (details to be published elsewhere), and for most strains it is actually very close to 15 days. When absolute time in the following is converted into number of generations, it will be on the premise that one generation equals 15 days. The length of adult life of the flies is rather short, probably not more than about two days on the average.

Sampling the populations. Egg samples are drawn from the populations by inserting a food vial one day earlier than scheduled, and leaving this vial for 24 hours' oviposition. The vial is then withdrawn from the box and its content of eggs is distributed to a sufficient number of food vials to allow the eggs to develop without serious competition. The gene frequencies of *cn* and *st* in populations otherwise wild type are

obtained by observing the frequency of mutant homozygotes in the sample. The gene frequency is then assumed to be the square root of the frequency of homozygotes. This calculation rests on the assumption that the genotypes in the sample are distributed binomially, *i.e.* that the genotypes occur in Hardy-Weinberg proportions. This assumption, which has been confirmed experimentally, will be fulfilled if the parent flies are randomly mated and selection within the sample is eliminated.

It is somewhat more laborious to estimate the *cn* or *st* frequencies in populations which are homozygous for a mutant allele in another locus controlling the production of the brown eye pigment, as all flies from such populations have bright red eyes. Such samples were treated as follows: All adult flies emerging in the sample were crossed individually to a tester stock homozygous for the allele whose frequency was to be estimated, but homozygous wild type in the locus in which the population is blocked by homozygosity with a recessive mutant. The genotype of the individual fly is determined from its offspring. A minimum of ten potentially segregating offspring was required to make the determination reasonably safe, but in the great majority of the flies the actual number of offspring was much higher. Thus, sample flies from blocked populations are classified for all three possible genotypes and the gene frequency is then calculated from the genotypic frequencies in the sample.

Replicated egg samples obtained by inserting two sample vials simultaneously into a population box have revealed that the sample variance between frequencies of identical genotypes in two samples is considerably greater than the variance expected based on the actual number of flies in the sample. The main reason for this, clearly, is that flies within a sample are not independent in origin. Rather, a sample consists of groups of offspring from the females that deposited the eggs in the sample vial. A number of comparisons between such replicate samples has shown that the variance is often five, and occasionally ten, times greater than the theoretical variance calculated from the size of the samples. This increased variance must be kept in mind when considering the results reported in the following.

III. RESULTS

1. Selection on st

a. *st on wild type Oregon "background"*

Three populations were initiated from hybrids between the *st*-strain and our *Oregon* wild type strain. The original gene-frequency of *st* in these populations was thus 50 %. It should be noticed that the "background", as used above and also for the following experiments, refers to the chromosomes introduced into the population from the st^+ strain from which the original hybrids were derived. Hence, the background in the sense used here comprises only half of the genome or, what is essential, the background differences between populations to be compared.

The development of these three populations as indicated by the samples is shown in Table 1, which gives the compositions of the samples in absolute numbers. The age of the populations at the date of sampling is given in days rounded off to the nearest multiple of ten.

It is seen from Table 1 that though the populations do not remain identical their development is very similar. For the present purpose the variation among initially identical populations is of interest only so far as it indicates the amount of uncertainty involved in estimating the selection under these specified conditions. From this point of view the inter-populational variance (or the intra-set variance), besides including egg sample variance, may be considered as due to random genetical drift in the broadest sense of this term. In other words, it would include not only the sampling variance necessarily involved in the transfer of genes from one generation to the next, but also possible genetical differences among the initial populations and statistical fluctuation superimposed on the directed selective drift (WRIGHT, 1956). Not much can be said about the relative importance of these three potentially major sources of variation among the populations, except that differences among the initial populations is probably of less importance in the sets in which the populations originated from hybrids between two rather inbred stocks.

As the populations, despite the differences pointed out, show the same general trend of selection against the *st* gene, and as we are here primarily concerned with the major features of selection of this allele, it is considered permissible to average the gene-frequencies of the three populations. These averages are given in the last column of Table 1, and Fig. 2 shows a selection curve based on these averaged values.

TABLE 1. *The frequency of st on Oregon "background" in three initially identical populations.*

Age in days	Population 58—46			Population 58—47			Population 58—48			Frequency of *st*-gene in			Average *st*-frequency
	st/*st*	total	*st*/*st* %	*st*/*st*	total	*st*/*st* %	*st*/*st*	total	*st*/*st* %	58—46	58—47	58—48	
0	—	—	25.00	—	—	25.00	—	—	25.00	50.00	50.00	50.00	50.00
120	137	648	21.14	189	508	37.20	136	621	21.90	45.97	60.99	46.79	51.25
150	36	228	15.78	77	259	29.72	25	150	16.66	39.72	54.51	40.81	45.01
200	30	161	18.63	87	328	26.52	26	192	13.54	43.16	51.49	36.79	43.81
250	53	350	15.14	107	450	23.77	48	397	12.09	38.91	48.75	34.77	40.81
350	124	404	30.69	41	275	14.90	4	160	2.50	55.39	38.60	15.81	36.60
410	22	818	2.68	102	786	12.97	26	468	5.55	16.37	36.01	23.55	25.31
720	4	157	2.54	4	205	1.95	9	122	7.37	15.93	13.96	27.14	19.01

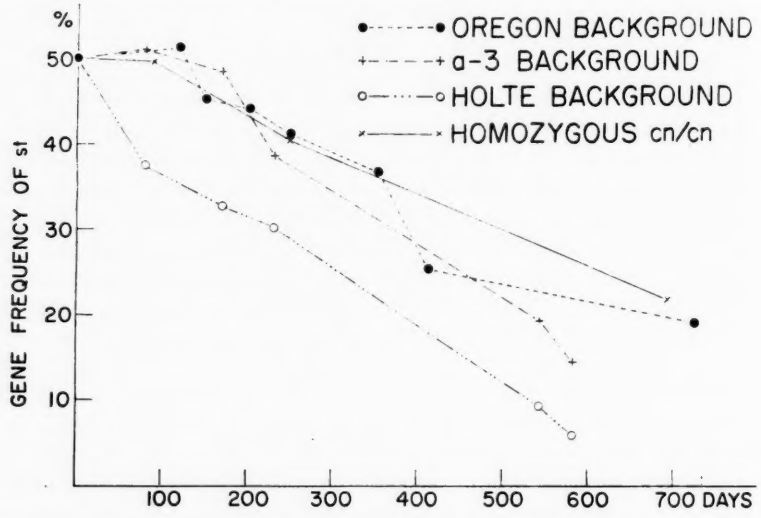

Fig. 2. The frequency of *st* in four different sets of populations. Each curve is based on the average frequencies in three separate populations.

From the table as well as from the figure it can safely be concluded that the *st* allele is consistently and significantly decreasing in frequency. The average frequency of the allele has gone down from the initial 50 % to about 22 % in 600 days, which equal approximately 40 generations.

b. *st on a-3 wild type "background"*

The changes in these populations, which were initiated from hybrids between the *st* strain and the *a-3* wild type strain, are given in Table 2, and the average selection curve is shown in Fig. 2. The development is essentially the same as in the above mentioned populations. The observed average decrease in the frequency of *st* is slightly greater as these populations have reached an average *st*-frequency of about 14 % in a little less than 600 days.

c. *st on Holte wild type "background"*

This set of populations was derived from hybrids between the *st* strain and our *Holte* wild type strain. As in the two former sets, the *st* allele is consistently decreasing in these populations (Table 3). The

TABLE 2. *The frequency of st on a-3 "background" in three initially identical populations.*

Age in days	Population 58—91			Population 58—92			Pupulation 58—93			Frequency of *st*-gene in			Average *st*-frequency
	st/st	total	*st/st* %	*st/st*	total	*st/st* %	*st/st*	total	*st/st* %	58—91	58—92	58—93	
0	—	—	25.00	—	—	25.00	—	—	25.00	50.00	50.00	50.00	50.00
80	191	768	24.86	287	1222	23.48	258	891	28.95	49.85	48.45	53.80	50.70
170	30	126	23.80	211	780	27.05	157	807	19.45	48.78	52.00	44.10	48.29
230	43	300	14.33	262	1319	19.86	86	788	10.91	37.85	44.56	32.87	38.42
540	5	168	2.97	55	626	8.78	3	252	1.19	17.23	29.63	10.90	19.25
580	23	852	2.70	56	801	6.99	1	816	0.12	16.43	26.43	0.34	14.40

TABLE 3. *The frequency of st on Holte "background" in three initially identical populations.*

Age in days	Population 58—88			Population 58—89			Population 58—90			Frequency of *st*-gene in			Average *st*- frequency
	st/st	total	*st/st* %	*st/st*	total	*st/st* %	*st/st*	total	*st/st* %	58—88	58—89	58—90	
0	—	—	25.00	—	—	25.00	—	—	25.00	50.00	50.00	50.00	50.00
80	129	775	16.64	112	851	13.16	73	586	12.45	40.79	36.27	35.28	37.44
170	34	484	7.02	12	127	9.44	87	530	16.41	26.49	30.72	40.50	32.57
230	36	627	5.74	54	508	10.62	50	453	11.03	23.95	32.58	33.21	29.91
540	4	605	0.66	6	665	0.90	44	650	6.76	0.81	0.95	26.00	9.25
580	1	483	0.21	7	742	0.94	8	319	2.51	0.44	0.96	15.84	5.74

TABLE 4. *The rate of change per 100 days* ($\triangle_{100}$) *averaged over the first 580 days of observation.*

	Individual populations			Average	Mean error
st on *Oregon*	5.83	4.49	4.22	4.85	0.50
st on *a—3*	5.78	4.06	8.56	6.13	1.24
st on *Holte*	8.54	8.45	5.88	7.62	0.87
st on wild type	—	—	—	6.20	0.62
st on *cn/cn*	—	4.75	3.32	4.04	0.71

average decline in the *st*-frequency is the greatest observed in any set. In a little less than 600 days an average frequency of about 6 % is reached.

d. *Comparisons of the wild type "background" sets*

In order to compare the selective forces operating in the three sets of *st* populations in wild type background, the rate of change in *st*-frequency per 100 days ($\triangle_{100}$) has been averaged over the first 580 days of observation for each population. In the case of the *Oregon* set this requires an estimate of the *st*-frequency reached at 580 days. This estimate has been obtained by simple linear interpolation.

The $\triangle_{100}$ values are set out in Table 4, together with the intra-set averages and the mean errors of these averages.

No significant differences can be demonstrated between the variances within sets. An analysis of variance reveals no significant increase of the variance among sets as compared to the variance within sets, the F-value being 2.5 with $^2/_6$ degrees of freedom. Thus, no significant difference has been established among sets. This may appear surprising when the average selection curves in Fig. 2 are considered. Furthermore, one may seriously question that some of the assumptions inherent in the analysis of variance are fulfilled in the present case. The conclusion reached is, nevertheless, appreciably substantiated when the individual populations on which the averages are based are taken into consideration. Thus, all three sets include at least one population which has reached an *st*-frequency within the range of 15—17 % at 580 days. The *Holte* set comprises two populations with an *st*-frequency of less than 1 % at 580 days, and a similar population is found in the *a-3* set. Finally, one population in the *Oregon* set, *viz.* 58—48, matches very

well with population 58—92 of the *a-3* set as far as the *st*-frequencies at 580 days are concerned.

Although a detailed analysis based on the individual selection curves over the entire range of observation might very likely reveal some significant differences among sets, we do not hesitate to pool the results from the three sets for the present purpose. The average $\triangle_{100}$ for all nine populations, together with its standard error, is given in the fourth row of Table 4.

e. *st in populations homozygous for* cn^s/cn^s

This set of populations was initiated from flies obtained as follows: Female F_1 hybrids from a cross between cn^s/cn^s and *st/st* were backcrossed to *st/st* males. In the F_2 generation about fifty per cent of the vials segregated flies with bright red eyes and wild type flies in the ratio 1:1. These *cinnabar* flies of constitution cn^s/cn^s, *st*/+ were isolated within a few hours of emergence and transferred to the population boxes. The initial frequency of *st* in this set was thus 50 %. The development of the populations is shown in Table 5 and Fig. 2.

Population 58—69 had to be discarded at the examination at 690 days because flies with wild type eyes occurred in the sample. No such cn^+ flies had been observed in sampling at 80 days and 250 days. The introduction of cn^+ may be due to either of two reasons, *viz.* backmutation of cn^s to cn^+ or contamination from the outside. As the population did not carry any other recessive mutants in homozygous condition, we are unable to distinguish between these possibilities. The sample derived from 58—69 population at 690 days is not included in the table.

The two remaining populations homozygous for cn^s agree with the populations on wild type backgrounds in showing a conspicuous decrease in the *st*-frequency which, on the average, has reached about 22 % in a little less than 700 days. Both the average population drawn in Fig. 2 and the individual populations set out in Table 5 make it obvious that the selection in the cn^s-blocked populations is not much different from that observed for the wild type population. The $\triangle_{100}$ values for the blocked populations are given in the last row of Table 4. A t-test of the difference between the average $\triangle_{100}$ for the wild type populations ($\triangle_{100}=6.20$) and the blocked populations ($\triangle_{100}=4.04$) is insignificant ($t=1.54$ with 9 degrees of freedom). This means that though the average $\triangle_{100}$ for the cn^s/cn^s populations is actually the lowest observed in any comparable set, the result is fully compatible with

TABLE 5. *The frequency of st in three initially identical populations which are cn/cn.*

Age in days	Pupulation 58—69			Population 58—70			Poputation 58—71			Frequency of *st*-gene in			Average *st*-frequency
	st/st	*st/+*	total	*st/st*	*st/+*	total	*st/st*	*st/+*	total	58—69	58—70	58—71	
0	—	—	—	—	—	—	—	—	—	50.00	50.00	50.00	50.00
80	49	110	203	28	83	156	40	80	152	51.23	44.55	52.63	49.47
250	11	58	103	27	71	144	11	27	63	38.83	43.40	38.88	40.37
690	—	—	—[1]	4	69	249	21	112	275	—	15.46	28.00	21.73

[1] Population 58—69 discarded at 690 days as it contained flies with wild type eyes.

TABLE 6. *The frequency of st in three initially identical populations homozygous v/v.*

Age in days	Population 58—61			Population 58—62			Population 58—63			Frequency of *st*-gene in			Average *st*-frequency
	st/st	*st/+*	total	*st/st*	*st/+*	tolal	*st/st*	*st/+*	total	58—61	58—62	58—63	
0	—	—	—	—	—	—	—	—	—	25.00	25.00	25.00	25.00
90	—	—	—	19	131	241	16	71	165	—	35.06	31.21	33.13
270	17	104	224	4	52	141	13	108	274	30.80	21.27	24.45	25.50
730	0	31	142	10	56	157	0	18	150	10.91	24.20	6.00	13.70

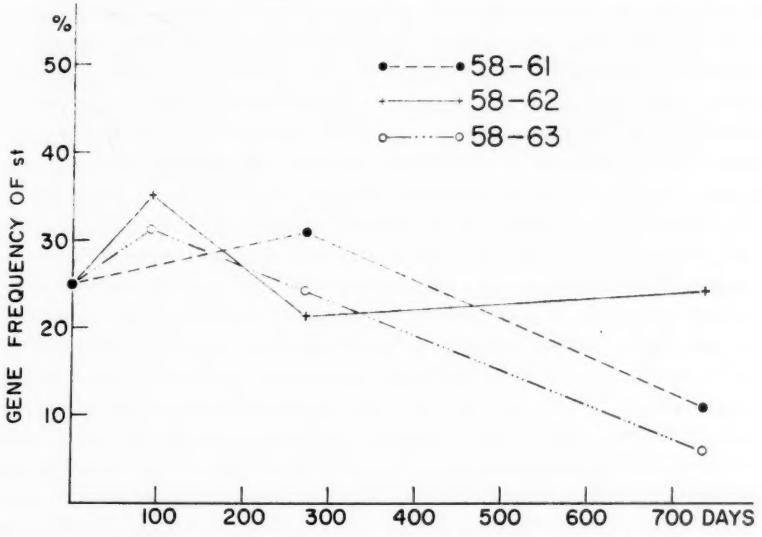

Fig. 3. The frequency of *st* in three initially identical populations homozygous for *v*.

the view that the blocking of biosynthesis at the step controlled by the *cn* locus has no effect whatever on the selective value of *st versus* st^+.

Hence, we are forced to conclude that, contrary to expectation, *selection against st is not suspended and not even relieved to a degree measurable in this experiment by substitution of* cn^+/cn^+ *with* cn^s/cn^s.

f. *st in populations homozygous for v*

This set was established in the following way: F_1 male hybrids between *v/v* females and *st/st* males were backcrossed to *v/v* females and the backcross progeny obtained was transferred to the population boxes. Thus, the initial frequency of *st* was 25 %. As the results obtained after two years vary conspicuously among populations (Table 6), the individual selection curves rather than the average curves are drawn in Fig. 3.

The average decrease during the two years is 11.3 %, yielding an average $\triangle_{100}$ equal to 1.51 with the $\triangle_{100}$s for the individual populations varying from almost zero to 2.6 %. These values are obviously not comparable to the $\triangle_{100}$s calculated from the entire frequency range of the wild type populations as the rate of change increases with initial

frequency. For this reason the average rates of change per hundred days for the three sets of wild type populations have been calculated from the changes observed at 25 % and downward. This gives the three values $\triangle_{100}=2.0$ for *Oregon*, $\triangle_{100}=7.9$ for *a-3*, and $\triangle_{100}=7.0$ for the *Holte* set. All these values exceed the average $\triangle_{100}$ for the populations homozygous for *v*, indicating that the substitution has been followed by a relief in the selection against *st*. However, a t-test shows that the difference between the overall average for the wild type sets and average for the *v/v* set is insignificant. A similar comparison has been made between the $\triangle_{100}$ values calculated for the range below 33 % in the two types of populations, as the *v/v* populations are consistent in showing an initial increase in the frequency of *st* to approximately 33 %. Neither could any significant difference be demonstrated in this way between the *v/v* populations and the wild type populations. Furthermore, it is questionable whether such comparisons are at all valid in the present case in which a glance at Fig. 3 leaves one with the impression that the *v/v* populations differ among themselves to a significant degree. After two years one of the *v/v* populations, 58—62, exhibits an *st*-frequency identical to the initial frequency, whereas the two remaining *v/v* populations show clear evidence of selection against *st*. Actually, the $\triangle_{100}$ of 58—63 is greater than the average $\triangle_{100}$ calculated for the range below 25 % in the Oregon wild type set. The two last mentioned populations indicate that substitution of v^+/v^+ with *v/v* is not invariably followed by a suspension of selection in the *st* locus.

Thus, we conclude that the data obtained show clearly that *substitution of v^+/v^+ with v/v is not invariably followed by a suspension of the selection on the st locus, but that there are some — though insignificant — indications in the data that the selection against the mutant allele of the st locus has been somewhat relieved.*

In passing it should be noted that the initial increase of frequency of the mutant allele is not confined to the *v/v* populations, but is true also for some other populations described in this paper, for instance 58—48 (Table 1). This phenomenon of initially very different selective forces operating during the first generations has been observed repeatedly by us when working with populations initiated with hybrids between strains which are not isogenic. Obviously, a randomization of genetic variation linked to the unit under observation has to be realized before the selective value of this unit will express itself.

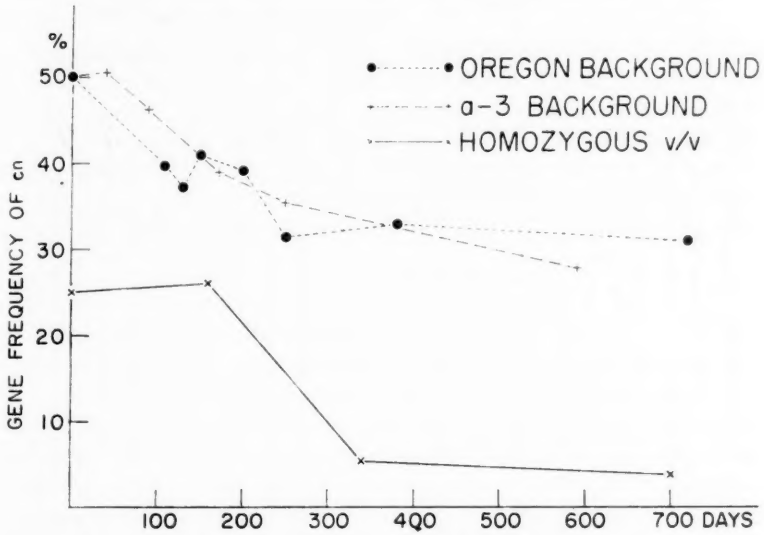

Fig. 4. The frequency of cn^s in three different sets of populations. Each curve is based on the average frequencies obtained in three populations.

2. Selection on cn^s

a. *cn^s on Oregon wild type "background"*

This set of populations was initiated from hybrids between the cn^s strain and our *Oregon* strain. The initial cn^s-frequency was thus 50 % and the frequency changes with time are shown in Table 7 and Fig. 4. The variation among populations is relatively minor and a clear picture of decrease in the cn^s frequency emerges. The decrease in about two years averages approximately 20 %, varying from 16 % to 22 %. There is some indication that the selection curves level out after 250 days at which time the average curve has already reached the frequency found after an additional 470 days of observation. This is compatible with the idea that the cn^s allele approaches an equilibrium frequency. However, the evidence for this is certainly not conclusive and the question can only be settled by further observation of the populations. At the present stage we may conclude that the cn^s allele is moderately selected against when the initial frequency is 50 %.

TABLE 7. *The frequency of cn^s on Oregon "background" in three initially identical populations.*

Age in days	Population 58—43			Population 58—44			Population 58—45			Frequency of *cn*-gene in			Average *cn*-frequency
	cn/cn	total	*cn/cn* %	*cn/cn*	total	*cn/cn* %	*cn/cn*	total	*cn/cn* %	58—43	58—44	58—45	
0	—	—	25.00	—	—	25.00	—	—	25.00	50.00	50.00	50.00	50.00
110	52	344	15.11	50	394	12.69	91	456	19.95	38.87	38.62	44.66	39.71
130	73	511	14.28	34	235	14.46	30	236	12.71	37.78	38.02	35.65	37.15
150	54	273	19.78	41	325	12.61	51	275	18.54	44.69	35.51	43.05	41.08
200	28	201	13.93	22	131	16.79	18	117	15.38	37.32	40.97	39.21	39.16
250	60	487	12.32	11	252	4.36	69	469	14.71	35.09	20.88	38.35	31.44
380	109	490	22.24	14	414	3.38	43	422	10.18	47.15	18.38	31.90	32.81
720	23	252	9.12	18	220	8.18	16	140	11.42	30.33	28.60	33.79	30.90

TABLE 8. *The frequency of cn^s on a-3 "background" in three initially identical populations.*

Age in days	Population 58—82			Population 58—83			Population 58—84			Frequency of *cn*-gene in			Average *cn*-frequency
	cn/cn	total	*cn/cn* %	*cn/cn*	total	*cn/cn* %	*cn/cn*	total	*cn/cn* %	58—82	58—83	58—84	
0	—	—	25.00	—	—	25.00	—	—	25.00	50.00	50.00	50.00	50.00
40	61	242	25.20	170	679	25.03	251	936	26.81	50.19	50.02	51.77	50.66
90	155	670	23.13	159	698	22.77	142	773	18.36	48.09	47.71	42.84	46.21
170	45	433	10.39	93	706	13.17	109	468	23.29	32.23	36.29	48.25	38.92
250	76	904	8.40	107	653	16.38	99	733	13.50	28.98	40.47	36.74	35.39
590	39	673	5.79	17	476	3.57	36	248	16.51	24.07	18.89	40.63	27.86

b. *cn^s on a-3 wild type "background"*

The set of populations derived from hybrids between cn^s and *a-3* also reveals a decrease of the cn^s allele (Table 8 and Fig. 4). The set as a whole does not indicate that an equilibrium frequency is approached, but it is worth noticing that population 58—84 has been remarkably stable during the last 500 days of observation. The average decrease in cn^s frequency is of the order of 22 % in about 600 days, the individual populations varying from a little less than 10 % to a little more than 31 %.

c. *cn^s in populations homozygous for v/v*

These populations were started by backcrossing male hybrids between *vermilion* females and cn^s males to the *vermilion* strain, and transferring the backcross progeny to the population boxes. The initial frequency of cn^s in these populations was then 25 %. As seen from Table 9 and Fig. 4, the change in cn^s frequency in this set is considerable. An average cn^s frequency of about 5 % is reached within a year. This value is further and consistently decreased to less than 4 % in the following year.

The average decrease during the first 590 days as estimated from the curves given in Fig. 4, is 21 % for the populations homozygous for *vermilion* and 16 % and 22 % for the two sets of wild type populations. A certain strength of selection would give rise to a greater gene frequency change in populations started at 50 % than in populations started at 25 %. As the *v/v* populations started at 25 % actually exhibit an average gene frequency change which equals that of one of the wild type sets and which exceeds that of the other wild type set, there is clear evidence that *the homozygosity of v/v has not at all relieved the selection against the cn^s allele.*

IV. DISCUSSION

Our attempt to predict the consequences of certain known genetical substitutions for the relative fitness of the mutant alleles of the *st* and *cn* loci has not met with success. The selective difference between wild type and mutant alleles of these loci was certainly not cancelled in populations made homozygous for mutant alleles of loci controlling a step in the biosynthetic sequence leading to the production of precursors for the *st* and *cn* loci. Not even a significant relief of selection could

TABLE 9. *Frequency of cn^s in three initially identical populations homozygous v/v.*

Age in days	Population 58—54			Population 58—55			Population 58—56			Frequency of *cn*-gene in			Average *cn*-frequency
	cn/cn	*cn/+*	total	*cn/cn*	*cn/+*	total	*cn/cn*	*cn/+*	total	58—54	58—55	58—56	
0	—	—	—	—	—	—	—	—	—	25.00	25.00	25.00	25.00
160	9	46	146	9	71	181	18	93	205	21.91	24.58	31.46	25.98
340	6	48	251	0	8	152	0	5	185	11.95	2.63	1.35	5.31
700	2	43	250	0	10	251	0	0	276	9.40	1.99	0.00	3.79

be demonstrated beyond doubt under these circumstances, although some indication was found of a slight relief in one case, *viz.* in the selection against *st* when the population is homozygous *v/v*. On the other hand, the selection against the *cn* mutant is intensified rather than relieved when the population is made homozygous for the same *v* allele.

Our predictions were based on a number of assumptions, firstly, about the action of the locus under observation, and secondly, about the action of the mutant alleles used in blocking the biosynthesis at a step prior to that controlled by the locus under observation.

1) We assumed that the locus under observation had only one primary function, *viz.* that of controlling a step in the sequence leading to brown pigment. This may not be true. If not, the advantage of the wild type allele even in the absence of precursor in the brown pigment sequence may be due to a primary pleiotropic effect. However, PARSONS and GREEN (1959) have recently presented evidence that the reduced fitness of flies homozygous for mutant alleles of the *v* locus is actually a consequence of the failure of these flies to perform the appropriate conversion in the sequence eventually leading to brown pigment. There is no reason why this should not be equally true for the *cn* and *st* alleles, and in that case primary pleiotropy is not responsible for the results found.

Granted that no primary pleiotropism is involved and that the synthesis is actually effectively blocked prior to the relevant step, it can still not be excluded that the locus is supplied with some other usable precursor from other metabolic channels. Another possibility is that the wild type allele under these circumstances is catalyzing the reverse reaction, resulting in the production of its own precursor which is then supposed to be of importance in some other metabolic pathway besides that of brown pigment production. Any of these possibilities, and we are aware that many more can be imagined, could account for the persistent superiority of the wild type allele.

2) The essential assumption made about the mutant alleles used for blocking the biosynthesis is that the allele is a true amorph, resulting in a complete block of biosynthesis in the homozygotes. This assumption may not be fulfilled. All mutants of the *vermilion* group are actually hypomorphs rather than amorphs to the extent that some brown pigment is always present in small amounts in mutant homozygotes (NOLTE, 1954). Apart from this general observation we have no further evidence that the cn^s allele used in the present study is a hypomorph

rather than an amorph. Thus, in combination with a suitable mutant allele of the *bw* locus, the cn^s yields white-eyed flies.

The *v* mutants, on the other hand, are known to fall into two categories (GREEN, 1954). One class of mutants, designated collectively as v^s, is modified to wild type in the presence of a non-allelic suppressor gene, *su-s*. Also, flies homozygous for a v^s mutant synthesize considerable amounts of brown pigment when the larvae are starved. The other class of *v* mutants, designated collectively v^u, is not modified by *su-s* and they do not respond to larval starvation. Under normal feeding conditions the two kinds of mutants accumulate like amounts of non-protein tryptophane and they may actually be amorphic. The v^s alleles are, however, clearly hypomorphs when the flies are raised on a sparse diet.

The *v* allele used in this study has been tested for reaction both to *su-s* and to starvation. In combination with *bw/bw* it reacts positively to both tests, and thus it belongs to the v^s class of *vermilion* mutants. As larval competition is very intense in the population boxes, starvation is in all likelihood the rule rather than the exception. In that case the *cn* and *st* loci have actually been provided with their appropriate precursors. This, of course, would fully explain why the substitution of v^+ with *v* has no effect on the selection in these loci. Experiments involving the use of a v^u mutant to test this explanation are in progress.

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SUMMARY

(1) It has been demonstrated that in artificial populations the mutant alleles of the *st* and the *cn* loci in *Drosophila melanogaster* are at a disadvantage compared to the wild type alleles. This was true in a number of wild type genetical backgrounds.

(2) The frequency changes of the same *st* and *cn* mutant alleles have been observed in populations made homozygous for other mutant alleles supposed to block the production of precursors to the biosynthetic steps controlled by the *st* and *cn* loci. Contrary to expectation, these substitu-

tions in the genetical background did not significantly alter the rates of frequency change of the *st* and *cn* mutants studied. These results are discussed.

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SOMATIC MUTATIONS OF FOUR GENES FOR ALBINISM IN BARLEY INDUCED BY X-RAYS AND ETHYL METHANE-SULPHONATE

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PROGRESS in our understanding of gene mutation requires the investigation of the mutation of specific genes (STADLER, 1954). This has been done in microorganisms, in forward- and back-mutation, as well as in *Drosophila* and mice. However, this approach has seldom been attempted in higher plants, except for the case where forward mutation was analysed in maize pollen treatments (STADLER, 1942).

This point seems to be of great importance when the possible selective effect of some mutagens is studied. It has been possible to demonstrate in *Drosophila* and barley, in experiments started in the forties (cf. FAHMY and FAHMY, 1957; GUSTAFSSON, 1960), that the mutation spectra can be shifted using different mutagens especially when comparing ionizing radiations and chemicals. Even differences between neutrons and X-rays have been observed in the mutagenic reaction of some loci for the *erectoid* character in barley (HAGBERG, GUSTAFSSON and EHRENBERG, 1958). Moreover, selective mutagenicity has been demonstrated in microorganisms (KÖLMARK and WESTERGAARD, 1953; DEMEREC, 1955; GILES, JR., 1951).

The writer has undertaken some experiments in order to obtain new information about the problem of selective mutagenicity, using barley strains heterozygous for a single gene controlling the normal development of chloroplats. The induced mutations in these strains can be scored somatically in the M_1 plants originated from the treated seeds or in the next generation in those M_2 spike progenies containing only chlorophyll deficient seedlings. Some results are reported here, using

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the somatic mutation analysis in four different genes for albinism in barley.

During the last few years, several studies have been published using, with more or less similar purposes, heterozygous strains in *Antirrhinum,* maize and barley (SPARROW and POND, 1956; STEIN and STEFFENSEN, 1959 a, b; and especially KOO, 1958; *cf.* SPARROW, BINNINGTON and POND, 1958, for early bibliography).

MATERIAL AND METHODS

The method of detecting somatic mutations is based on the assumption that if a plant is heterozygous for a gene, *e.g.* controlling a character as albinism, *Aa*, any mutation producing a change of $A \rightarrow a$ or a deletion of *A*, will give the recessive phenotype, *aa* or *a*. A cell where such an event occurs, as well as its daughter cells, will then show a phenotype differing from the rest of the plant. If a meristematic cell of a leaf primordium is affected and there is no selection against it, a streak or a spot of tissue will be the result; in barley, as in other *Gramineae,* one streak will be formed in consequence.

As a normal plant probably carries a large number of indispensable genes controlling chloroplast development, the process $AA \rightarrow aa$ is possible in the homozygous as well as in the heterozygous plants. If mutations are independent events, the frequency of such a simultaneous occurrence will be much lower in a homozygous cell than in a heterozygous one. In fact, in irradiated diploid barley it would be a function of the square of the dose.

Other disturbances in scoring the mutation rate of individual loci would be plastid mutations, phenocopies, etc. Lastly, the normal tissue in the neighbourhood of the mutated sector may provide metabolites which makes it indistinguishable from the rest. Consequently, in order to determine a mutation rate on a per locus basis using the somatic mutation frequency, it is necessary to compare the streak frequency in the leaves of heterozygous and homozygous plants.

If we call H the streak frequency per leaf in heterozygous plants and D the same in the corresponding homozygous ones, n the total number of loci controlling normal chloroplast development, $\bar{m}$ the mean mutation frequency per leaf for the n genes per unit dose, $\bar{m}_1$ the same for n-1 genes and, finally, m_a the mutation frequency per leaf for a certain gene per unit dose, we will have:

$$D = n\bar{m}^2 + T$$
$$H = m_a + (n-1)\bar{m}_1^2 + T$$

T represents all the effects due to plastid mutations, phenocopies, etc., following an unknown function but assumed to be identical for the heterozygous and the homozygous plants. It can also be presumed that m and m_1 differ only very little and that consequently their difference can be neglected. Then, the difference $\triangle$ between H and D becomes:

$$\triangle = H - D = m_a - m^2 \qquad (1)$$

If the difference $\triangle$ does not differ significantly from 0, we can conclude that the mutation or streak frequency per leaf for this particular gene is also 0 or too low for detection with the resolving power of the method, which depends on the number of plants and leaves tested.

Four different strains heterozygous for one pair of genes for albinism have been used and their origins and characteristics are shown in Table 1. They were selected for having a clear-cut segretion ratio of 3 normals: 1 albina seedlings in F_2 and for being representative of three different genetic backgrounds. Besides, they were originally induced by different treatments.

The progeny of homozygous and heterozygous plants was treated under exactly the same conditions. From the progeny of the heterozygous plants, one-half is expected to be heterozygous, one-fourth homozygous dominant and one-fourth *albina* dying at an early stage. As it is not possible to distinguish the normal green plants in homo- and heterozygotes, it is necessary to calculate H (formula 1) using the data gathered in the heterozygous progeny (Het), as it follows:

$$H = 1.5\ (Het - D/3) \qquad (2)$$

The mutagens used in this study were X-rays and ethyl methanesulphonate (for brevity EMS). This chemical was selected since it has

TABLE 1. *Details about the four strains used in this study.*

Locus	Mother line	Located on	Origin of the recessive gene	Supplied by
alb_1	Trebi (6-row)	Chromosome 5	Spontaneous	D. ROBERTSON
alb_{20}	M. Heda (2-row)	Other than chromosome 5	Gamma-ray treatment	E. A. FAVRET
alb_{14}	Bonus (2-row)	—	X-ray treatment	D. VON WETTSTEIN
alb_{15}	Bonus (2-row)	—	Neutron treatment	D. VON WETTSTEIN

proved to be a very efficient and effective mutagen in barley (HESLOT *et al.*, 1959; HESLOT, 1960; EHRENBERG, 1960) as well as in *Neurospora* (WESTERGAARD, 1957); related compounds are also mutagenic in *Drosophila* (FAHMY and FAHMY, 1956). The X-ray treatments were performed at 175 and 240 KV and 15 mA, delivering 800—1 000 r/min., the seeds having a water content of about 11 %. The EMS treatments were made by soaking the seeds in solutions of different concentrations for 24 hours with continuous shaking and using 10 times more solution than the corresponding volume of the seeds. After the treatment, the seeds were washed thoroughly in tap water and sown within the next 12 hours.

Treated and control plants were grown in pots or boxes under greenhouse conditions. They were supplemented with nutrient solutions and artificial light when necessary, in order to obtain a normal development of the plant. Diseases which could prevent the accurate scoring of the streaks were controlled.

When the plants developed their 6th leaf in full length, counting from the bottom (happening 45 to 60 days after sowing), they were carefully checked for chlorophyll deficient streaks taking all the length of the blade. As a matter of fact, only a few such streaks appear in the 3rd leaf and none in the first two. Leaves 4, 5, 6, and sometimes 7 and/or the first leaf of the first secondary shoot, were considered. None of the streaks detected in a leaf was observed in more distal ones; consequently, each streak was considered a mutation (the term *mutation* used in a broad sense). Other details concerning leaf growth will be published elsewhere (FAVRET and PACHECO, in preparation).

RESULTS AND CONCLUSIONS

All the data collected for every treatment have been pooled and presented in Table 2. The difference between the mutation frequency of heterozygous and homozygous plants was analysed statistically using the STUDENT-FISHER *t*-method, the variance of the heterozygous strain calculated on $^2/_3$ of the number of tested leaves.

Spontaneous mutation occurs in a very low frequency, 1 streak on 3 049 leaves. On the other hand, X-ray and EMS treatments increase the mean frequency from 30 to 250 times in the homozygous strains. The chemical is evidently more mutagenic than the X-rays if the homozygous strains are compared (Fig. 1), both frequencies following an exponential increase when plotted against dose or concentration.

TABLE 2. *Streak frequencies obtained in the treatments with X-rays and ethyl methanesulphonate.*

Treatment	Locus	Heterozygous plants				Homozygous plants			△ (form. 1) $\times 10^{-3}$ (H-D)	t-value	P =
		Leaves tested	Streaks observed	Streak freq./ leaf $\times 10^{-3}$ (Het)	Calculated (form. 2) $\times 10^{-3}$ (H)	Leaves tested	Streaks observed	Streak freq./ leaf $\times 10^{-3}$ (D)			
Control	—	2 589	0	0.0	0.0	460	1	2.1	− 2.1	±0.8	> 0.4
X-rays dry seeds 6 to 24 Kr	alb_t	1 792	98	54.6	76.9	600	6	10.0	+ 66.9	±8.3	< 0.001***
	alb_{20}	268	18	67.1	82.5	413	15	36.3	+ 46.2	±2.0	< 0.05*
	alb_{14}	384	21	54.9	75.7	304	4	13.1	+ 62.6	±3.4	< 0.001***
	alb_{15}	330	22	66.6	93.3	„	„	„	+ 80.2	±3.8	< 0.001***
EMS soaking seeds 24 hrs.	alb_t	561	81	144.0	193.6	470	21	44.6	+149.0	±6.2	< 0.001***
	alb_{20}	708	42	59.3	61.9	760	41	53.9	+ 8.0	±0.5	> 0.6
	alb_{14}	283	20	70.7	69.0	337	25	74.0	− 5.0	±0.2	> 0.8
	alb_{15}	331	24	72.0	70.9	„	„	„	− 3.1	±0.1	> 0.9

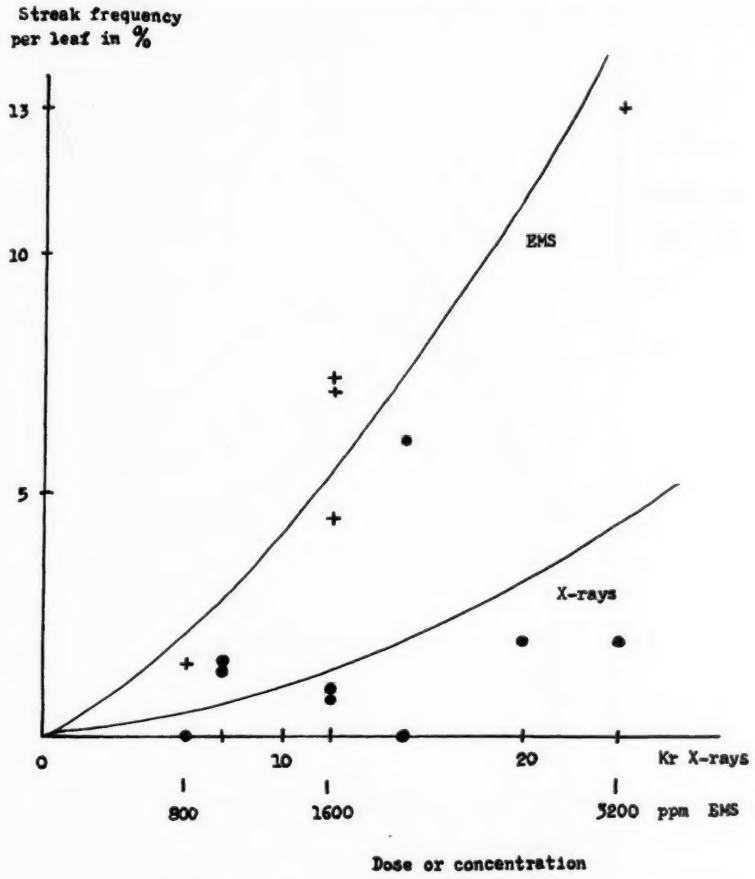

Fig. 1. Streak frequency per leaf in X-ray and EMS treatments of homozygous plants, plotted against dose or concentration.

However, the treatments with both mutagens are not strictly comparable since the seeds were in different conditions. Besides, unpublished data on X-ray treatments of pregerminated seeds show a much higher mutation frequency than on dry ones.

If the seed damage is considered, measured as the number of surviving plants at the 6th leaf stage, EMS proves to be more efficient in inducing streaks than X-rays, as it is observed in Fig. 2, where mutation

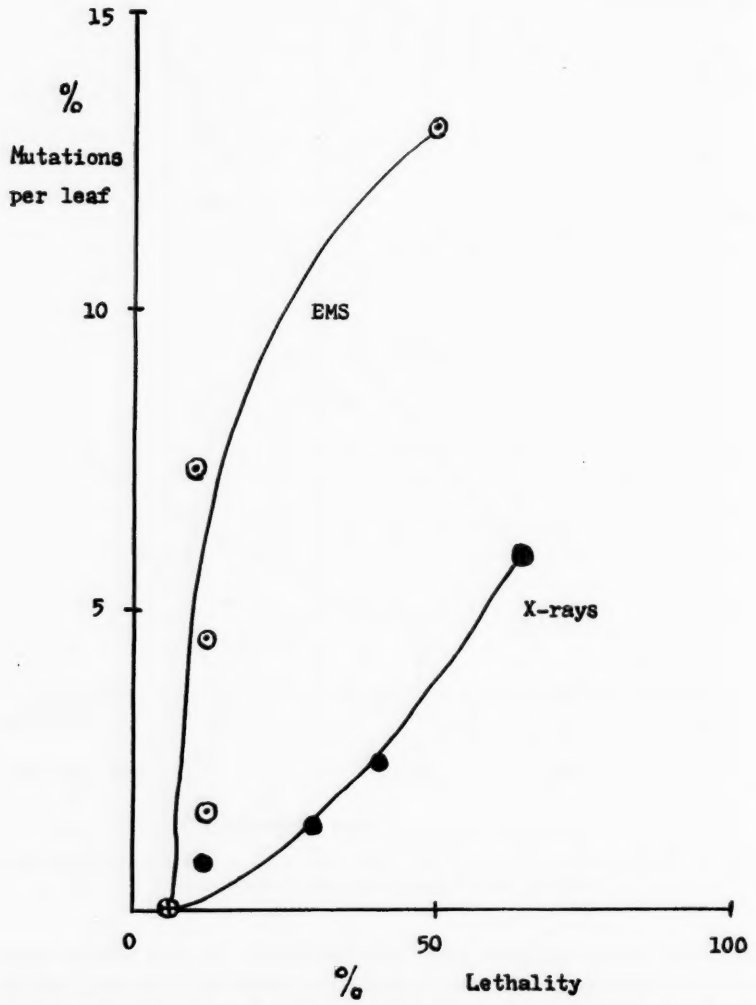

Fig. 2. Relationship of lethality and mutation rate in treatments with X-rays and ethyl methanesulphonate. Homozygous strains only.

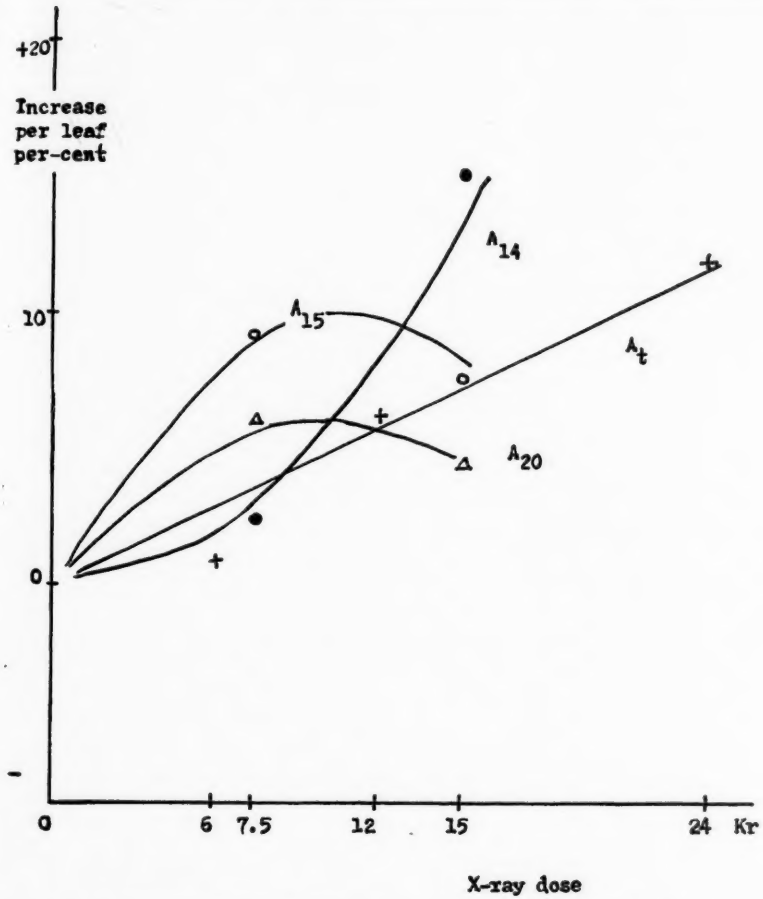

Fig. 3. Mutation rates of four genes $A \rightarrow a$ plotted against X-ray dose.

frequency has been correlated to lethality. This observation is in accord with the data obtained through the genetical analysis of X_2 generations (HESLOT, 1960; EHRENBERG, GUSTAFSSON and co-workers, unpubl. data).

The comparison of the single locus reactions (Table 2, column $\triangle$; Fig. 3 and 4) indicates that the four forward mutation rates are rather similar when X-rays are used. The reactions to EMS are strikingly dif-

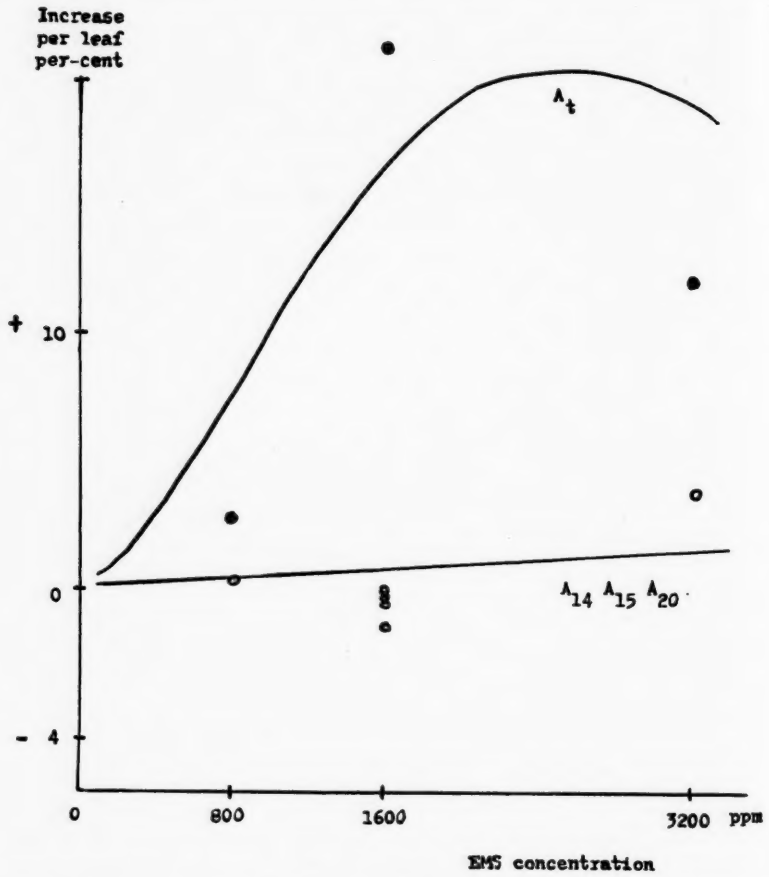

Fig. 4. Mutation rates of four genes plotted against the concentration of ethyl methanesulphonate.

ferent, the alb_t locus being the single one mutating. The other three gene mutation rates remained unaltered by the EMS treatments, the differences between the homozygous and heterozygous strains varying insignificantly around 0, with minus and plus values as expected on a random basis. Consequently, a selective mutagenicity can be attributed to EMS in comparison to X-rays. The ionizing radiations would have

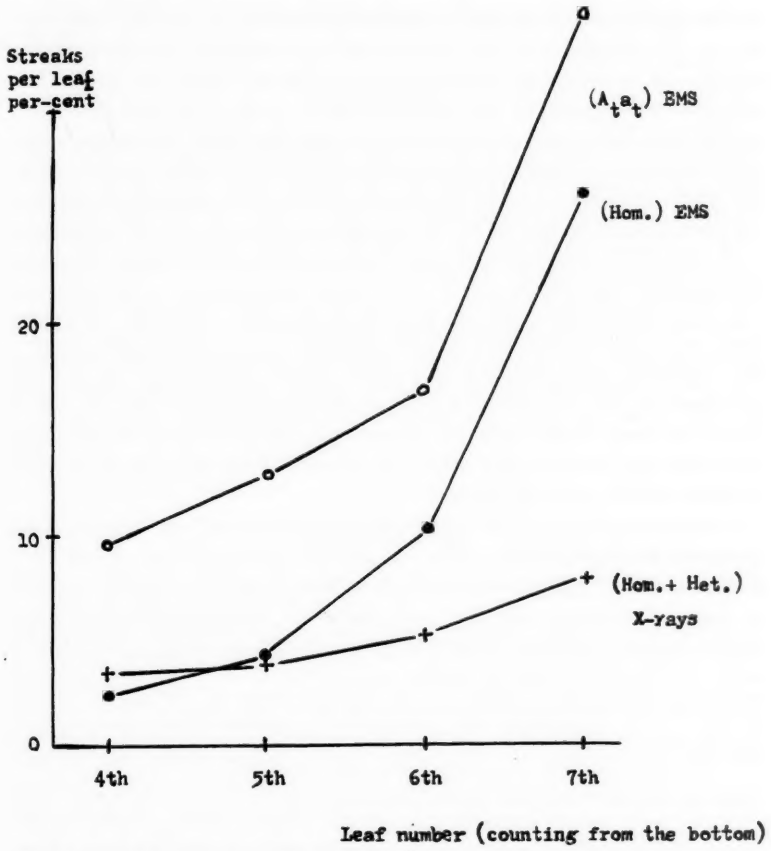

Fig. 5. Streak frequency in different leaves, X-ray and ethyl methanesulphonate treatments.

an unselective activity (see also KOO, 1958, on the analysis of loci a_n and x_c on barley irradiated with X-rays).

If plotted against dose or concentration, the mutation frequency per locus seems to increase linearly, though a drop is observed with the highest used concentration of EMS. The maximum increase would be around 20 % per leaf.

Another difference between EMS and X-rays is observed if the mutation frequency is compared in different leaves (Fig. 5). While the X-

ray treatments show an almost similar frequency in the four leaf types (4—7), the frequency in the EMS treatments increases in upper leaves, both in the homozygous and heterozygous plants. This can be due to a different distribution of the chemical in the embryo or to a difference in cell sensitivity; but, as streaks not extending over the entire length of the blade are sometimes observed in the chemical treatments, especially in the 4th leaf, it could also be presumed that such effects are produced by a delayed action of the mutagen or by a lag in the expression of the mutated gene. No such streaks have been observed in the X-ray treatments of dry seeds.

It is, incidentally, interesting to state here the different activity of EMS and ionizing radiations on chromosome damage (unpubl. results). The chemical produces in barley none or few chomosome breaks and no chromosome exchanges. A marked delay of cell division as well as the occurrence of chromosome erosion are the effects observed. Ionizing radiations, on the other hand, act in part by the production of chromosome breaks and exchanges.

A comprehensive review on chemical mutagenesis has recently been presented by EHRENBERG (1960). In general, chemicals act on the chromosomes producing heterochromatin abnormalities especially, not only in the case of EMS, but also with myleran (MOUTSCHEN and MOUTSCHEN-DAHMEN, 1958 a), ethyleneimine (the same writers, unpubl. results), 8-ethoxycaffeine (KIHLMAN and LEVAN, 1951; MOUTSCHEN and MOUTSCHEN-DAHMEN, 1958 b) and maleic hydrazide (MCLEISH, 1953), though every compound shows some characteristic effects. It would then be possible that the localization of the genes within the chromosomes in relation to the neighbouring heterochromatin had some importance and would explain the selective mutagenicity of those chemicals. They could represent true *point mutations*. On the other hand, the genetical consequences of the ionizing radiations are more likely gene deficiencies and duplications, occurring at random along the chromosomes.

The main conclusions of our studies are (1) the usefulness of the method based on somatic mutations for detecting frequencies assigned to specific loci, and (2) the demonstration that EMS has a selective mutagenic effect.

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SUMMARY

The forward mutation frequencies of four genes for albinism in barley have been studied by means of treatments with X-rays and ethyl methanesulphonate. The method consisted in scoring the number of chlorophyll deficient streaks on leaves of the M_1 plants, comparing heterozygous and homozygous strains. The genes analysed were alb_t, alb_{20}, alb_{14}, and alb_{18}. All of them mutated in X-ray treatments with similar frequency. The ethyl methanesulphonate, on the other hand, gave mutations in alb_t and none in the other three genes. It is concluded that ethyl methanesulphonate has a selective mutagenic effect. This chemical is a more efficient as well as a more effective mutagen than X-rays.

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QUALITATIVE AND QUANTITATIVE ANALYSES OF EYE PIGMENTS AND PTERIDINES IN BACK-MUTATIONS OF THE MUTANT w^a IN DROSOPHILA MELANOGASTER

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I. INTRODUCTION

INTENSIVE studies on higher organisms as well as on micro-organisms have been devoted to the question whether or not reverse or back mutations are able to completely restore the function of the wild-type gene from which they originated. Where morphological characters are concerned, subjective decisions are often involved in the estimation of the completeness of the phenotypic reversion. For an objective analysis it is necessary to choose a mutant whose phenotype can be quantitatively as well as qualitatively separated from that of its wild-type allele. Further, the mutant must be genetically well defined, such that reverse mutations can be objectively distinguished from other causes of phenotypic reversion, such as mutations to non-allelic supressor genes, phenocopies, crossing over events, gene conversions, etc. All available criteria must agree in showing that the "mutational event" is an intragenic one, confined to the locus under observation. Still, it may not be possible to discriminate "true reversions" from all kinds of supressor gene mutations, particularly those tightly linked to the mutant locus, or included within the same pseudoallelic region. However, such events are very improbable, and should be practically ruled out if several reversions, both spontaneous and induced, are derived from the same mutant.

Not only would a detailed quantitative analysis of gene function provide information about the completeness of the mutational reversion,

but it also might serve as a basis for discriminating between wild-type alleles previously regarded as identical, so-called isoalleles (STERN and SCHAEFFER, 1943; GREEN, 1959).

Especially suitable for quantitative studies of gene function in *Drosophila melanogaster* are those mutants, which affect the production of eye pigments and fluorescent substances so easily separated by paper chromatography (HADORN, 1959). In a series of investigations on *Drosophila* it has been shown that most of the fluorescent substances as well as the red eye pigment are pteridine derivates (FORREST and MITCHELL, 1954 a and b; VISCONTINI *et al.*, 1955), many of which have been chemically identified. The various pigmented mutants of the pseudoallelic *white* region in the X-chromosome of *Drosophila melanogaster* differ in their pattern of fluorescent substances (RASMUSON, unpublished) and also in their amount of visible eye pigments. Since this region is also genetically well known, a study of reversions from some *w*-mutant might give conclusive results concerning the completeness of reverse mutations, and the occurrence of isoalleles.

The present investigation is an analysis of a series of reverse mutations obtained from the *apricot* gene in the *w*-locus. It includes a quantitative estimation of both red and brown pigments and some pteridines.

Material

The stocks and w^a reversions studied are listed in Table 1. All reversions arose spontaneously or following X-ray treatment from stocks containing w^a. One, No. 4, was originally supposed to be a spontaneous reversion from the *eosin* gene in the stock *y* w^e *ec* in Stockholm. However, subsequent tests with the specific supressor of w^a, *su-*w^a (X-0.05) have shown that the eye color mutant of this stock is w^a, not w^e, since the mutant is suppressed by *su-*w^a.

The phenotypic appearance of the reversions is near wild-type for all reversions excepting numbers 3, 6 and 8. The latter three have a distinctly maroon coloration which readily permits their separation from wild-type. All reversions were tested for the possibility that they are not true reversions but rather w^a combined with a suppressor mutation. In five of the reversions 1, 2, 5, 6 and 7 if a suppressor mutation is involved, it must be a dominant suppressor. Each reversion was recovered in females; 1, 5, 6 in crosses where the females should be homozygous for w^a, 2 and 7 where the females should be heterozygous for w^a and a *w* mutant. The rarity of dominant suppressors suggests that it is un-

TABLE 1. *Stocks and w^a reversions studied.*

Genotype and Symbol	Origin [1]	Source
Canton-S wild-type — +		M. M. GREEN
White-apricot — w^a	s	E. B. LEWIS
w^a-57i11=R-1	s	M. M. GREEN
w^a-58l12=R-2	s	M. M. GREEN
w^a-M=R-3	s	J. C. MOSSIGE
w^a-Mc=R-4	s	M. E. MACKENDRICK
w^a-59k1=R-5	x	M. M. GREEN
w^a-59k9=R-6	x	M. M. GREEN
w^a-59k13=R-7	s	M. M. GREEN
w^a-60a5=R-8	s	E. SHERWOOD

[1] s=spontaneous; x=x-ray induced.

likely that they can account for the reversions studied. Four of the reversions, 1, 3, 5 and 6, were tested for recombination with the mutant w^{ch}, a pseudoallele of w^a. Assuming the reversions to be changes of the w^a mutant, free of structural change, each would be expected to recombine with w^{ch} as does w^a. These expectations were fulfilled and recombination between w^{ch} and the reversions was found. Therefore it is concluded that the reversions represent "point" mutational changes. The alleles have been studied in homozygous or hemizygous condition. The flies were cultured on standard cornmeal-molasses-agar-yeast medium at $25 \pm 0.5^\circ$ C. By means of several transfers over-crowding has been avoided. At the time of eclosion the cultures were emptied with short intervals and after having been aged for 3—4 days imagos were deep-frozen and stored in this condition until the chemical analysis.

Analytical methods

For the quantitative determination of the eye pigments the spectrophotometric method of EPHRUSSI and HEROLD (1944) was followed with minor modifications. Homozygous females were used for all estimations of eye pigments. The flies were decapitated and an incision was made between the eyes without damaging them, thereby guaranteeing a complete and more rapid extraction of the pigments. The red pigment was first extracted, then the brown. To extract the red pigment 30 heads (for w^a 45) were immensed in boiled distilled water, made slightly acid by addition of one drop of 18.5 % HCl, for 48 hours. The brown pig-

ment was then extracted for 72 hours in acid methanol (1 ml conc. HCl per 100 ml methanol). Since the brown pigment functions as a redox-system a drop of 1 % H_2O_2-solution was added to this extract 1 hour before the measurements. The photometric measurements were performed with a Beckman DU spectrophotometer at a wave length of 480 mμ for the red and 470 mμ for the brown pigment. A constant slit width of 0.015 mm was used. The intensity was measured as the extinction, log I_o/I, and adjusted to correspond to 10 heads per ml of solvent.

The red eye pigment is composed of at least three closely related compounds, the drosopterins, which can be separated by paper chromatography, using isopropyl alcohol/ammonium-acetate as solvent (VISCONTINI, HADORN and KARRER, 1957). The exact conditions are given in Table 2. For a quantitative estimation the outlines of the spots after development were drawn in UV-light. The encircled pieces of paper were cut out and the substances eluated in acid water. The intensity of the solutions was measured in a Beckman-DU at 480 mμ. The extraction of the red pigment was made from a homogenate of 100 heads in 1 ml of acid 50 % methanol. 0.4 ml of the supernatant after centrifugation was deposited on Whatman 1 paper for the chromatographic development. The eluation of the spots was made in 3 ml water. The intensity found thus corresponds to $100 \times 0.4/3 = 13.3$ heads per ml. To obtain a more complete eluation of the substances, the cut out papers have sometimes been eluated twice and the estimates added together. This was necessary for the wild-type, which contained the greatest amount of pigments.

For the estimation of the fluorescent substances either heads, abdomens or intact animals were homogenized in 50 % methanol, 50 animals per ml. The homogenates were centrifuged and the supernatant decanted off. For the chromatographic development a narrow line of the extract, one or two cm long, was deposited on Whatman 1 paper. The amount deposited differed, and depended on the genotype used and the substance under observation. Exact details are given in the tables. Since some of the fluorescent substances are unstable in solution the deposition on the paper and the desiccation of the extracts was made immediately following homogenization. The technics used for the chromatographic separation are given in Table 2. Chromatograms were developed in complete darkness. Strips of the papers containing the fluorescent spots were cut out under UV-light, and the intensities were recorded in an Analytrol Spinco, Model RA, especially rebuilt for the purpose. With the filter combination used it records all kind of fluor-

TABLE 2. *Methods for chromatographic separation and estimation of fluorescent substances. Descending development in total darkness at 25° C.*

Solvent	Separated compounds	Time of development	Method for estimation of intensity
1. butanol /5N acetic acid 2 : 1	isoxanthopterin, IX HB-pteridine sepiapteridine, SP	18 hrs	Analytrol (SP visual estimation only)
2. butyl ether/chloroform/ /acetic acid/H_2O 20 : 20 : 20 : 2.5	isoxanthopterin, IX 2-amino-4-hydroxy-pteridine, HB_1 biopterin, HB_2	18 hrs	Analytrol
3. isopropyl alchohol/2 % NH_4-acetate 1 : 1	neodrosopterin drosopterin isodrosopterin	10 hrs	Beckman DU 480 mμ

escence except the yellow one. The Analytrol draws a curve for the intensity with simultaneous integration, whereby the area under the peak can be directly read off. A description of the apparatus will be made elsewhere (BJÖRKMAN, RASMUSON and BERGERON, in preparation).

II. RESULTS

Eye pigments

The spectrophotometric estimation of the amounts of red and brown pigments are shown in Table 3 and Fig. 1. The main features are the following:

The reversions vary in their amounts of red as well as brown pigment. Two pairs, however, seem to be identical, viz. *w*^{a}R59k9 with *w*^{a}R60a5 and *w*^{a}R57i11 with *w*^{a}R59k13.

In all the reversions the amount of red pigment is less than that of the wild-type, in fact less than 40 % of its intensity, and two of them, the identical *w*^{a}R59k9 and *w*^{a}R60a5, have not increased the amount at all, but remain at the *apricot* level. For most of the reversions brown pigment equivalent to that of Canton-S is found; only *w*^{a}R59k9, *w*^{a}R60a5 and *w*^{a}RM have significantly less. *w*^{a}R58l12 and *w*^{a}RMc have higher amounts than Canton-S, but the excess is small and significant at the 0.05 level only for *w*^{a}R58l12. Even the least intense reversions have in-

TABLE 3. *Estimates of red and brown eye pigments in different genotypes. The intensities correspond to a concentration of 10 heads per ml.*

Genotype	Number of determinations	Red pigment		Brown pigment	
		$100 \times \log I_0/I$	% of wild type	$100 \times \log I_0/I$	% of wild type
Canton-S	5	59.02±0.52	100	10.36±0.30	100
w^a	5	3.52±0.15	6	1.82±0.07	18
Reversions:					
No. 1 w^aR57i11	5	19.98±0.47	34	9.14±0.69	88
No. 2 w^aR58l12	5	21.74±0.73	37	12.34±0.82	119
No. 3 w^aRM	5	5.98±0.51	10	7.26±0.54	70
No. 4 w^aRMc	5	19.18±0.67	32	11.46±0.45	111
No. 5 w^aR59k1	10 (5 for BP)	15.74±0.46	27	8.22±0.31	79
No. 6 w^aR59k9	10 (5 for BP)	3.70±0.32	6	4.98±0.26	48
No. 7 w^aR59k13	9 (6 for BP)	20.79±0.45	35	9.03±0.55	87
No. 8 w^aR60a5	6	3.45±0.16	6	4.82±0.25	47

creased the amount of brown pigment to more than the double that of w^a.

Within the reversions, a positive correlation between the amount of red and brown pigment is obvious ($r=0.895$; $P<0.01$). The regressions

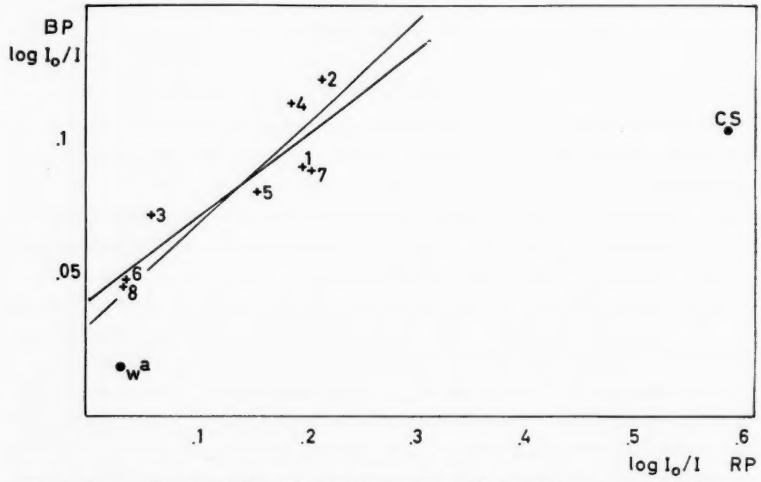

Fig. 1. Amounts of red (X-axis) and brown (Y-axis) eye pigments in wild-type, *apricot* and eight reversions from w^a.

TABLE 4. *Estimates of the drosopterins after chromatographic separation of the red pigment.*

Genotype	Number of determinations	$100 \times \log I_0/I$						Total DP
		Spot No. 1 neodrosopterin		Spot No. 2 drosopterin		Spot No. 3 isodrosopterin		
		Intensity	% of total DP	Intensity	% of total DP	Intensity	% of total DP	
Canton-S	3	7.60±0.29	18.3	19.77±0.90	47.7	14.07±0.43	34.0	41.43±1.62
Reversions:								
w^{a}R57i11	8	2.58±0.11	18.3	7.90±0.32	56.2	3.59±0.17	25.5	14.06±0.44
w^{a}R58l12	7	2.23±0.19	16.4	8.39±0.23	61.6	2.99±0.29	22.0	13.60±0.50
w^{a}RM	8	1.73±0.10	26.2	2.45±0.17	37.3	2.39±0.19	36.5	6.56±0.30
w^{a}RMc	7	3.19±0.09	18.2	9.99±0.27	56.9	4.37±0.17	24.9	17.54±0.34
w^{a}R59k13	4	3.85±0.40	22.2	10.10±0.37	58.1	3.43±0.19	19.7	17.38±0.55
w^{a}R60a5	4	2.15±0.21	38.2	2.13±0.05	37.8	1.35±0.12	24.0	5.63±0.25

of brown on red pigment and vice versa are indicated on Fig. 1. These regression lines do not pass in the vicinity of the original stock *w*^{a}, nor of the wild-type. By comparison, the relative amounts of red and brown pigment of the reversions show they are not simply intermediates between *w*^{a} and the wild-type.

Chromatographic analyses

A. *The drosopterins*

The components of the red eye pigment, the drosopterins, have been chromatographically separated (see Table 2) and estimated in the Canton-S genotype and in 6 of the reversions. Because of its low intensity of red pigment, the mutant *w*^{a} genotype was not included in this analysis. The two reversions, *w*^{a}R59k1 and *w*^{a}R59k9 were not available at that time. The results are shown in Table 4.

All three components of the red pigment are affected when the total amount is increased in the reversions. The relative composition, however, varies. The four most intense reversions form a rather homogeneous group where drosopterin predominates, whereas wild-type and *w*^{a}RM have more of isodrosopterin and *w*^{a}R60a5 more of neodrosopterin. In these two reversions where the total amount is very low, the estimation of the components is difficult and may be unreliable.

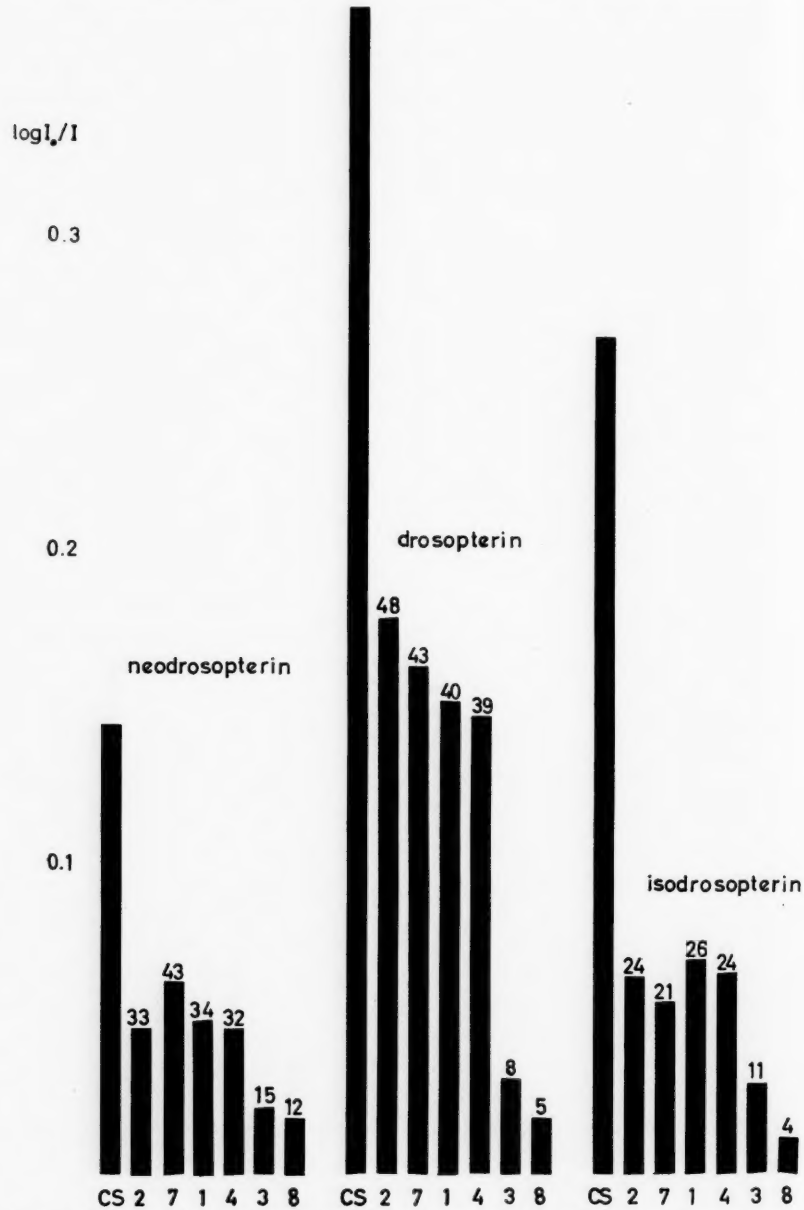

Fig. 2. Corrected (see text) estimates of the drosopterins in wild-type and reversions, arranged after decreasing amounts of total red pigments.
% of wild-type indicated on top of the columns.

A comparison between the totals in Table 4 and the estimates of total red pigment given in Table 3, reveals that the elution of the separated components has not been quite complete when the genotypes with higher concentrations are concerned, not even for the wild-types which has been eluted twice. To correct for this loss, the values of Table 4 have been adjusted to a total which corresponds to 13.3 heads per ml according to Table 3 (multiplication with a factor obtained as 1.33×RP/total DP for each genotype). Assuming that no difference exists among spots in their capacity of elution, this procedure should give a reliable picture of the relationship among genotypes. This is graphically illustrated in Fig. 2. When the genotypes are arranged in order of decreasing amount of total red pigment, the same decreasing order recurs in spot No. 2, the drosopterin, and also, with some minor irregularities, for the most intense reversions in the two other spots. No component reaches 50 % of the quantities found in the wild-type. The estimation of the components of the red pigment indicates that the formation of these compounds is a coupled process, the equilibrium of which is not altered in the reversions.

B. *The fluorescent substances*

The chromatographic methods used for the separation of the fluorescent substances are given in Table 2. While many different solvents have been used for different purposes, in this investigation the main effort has been the discrimination of genotypes by means of quantitative estimations on the various fluorescent substances. Therefore, in the choice of methods more stress has been placed on a good separation rather than on the identification of substances by comparison with other investigations. The names given to the substances in Table 2 are presumptive and should not be assumed to be unequivocally identified.

The following substances, known to be present in *Drosophila melanogaster*, have been considered:

isoxanthopterin, IX, which is easily recognized by its deep blue fluorescence. It occurs in eyes as well as in gonads of most genotypes, and is especially concentrated to the testes;

xanthopterin, X, has a blue-green fluorescence and has been observed only after development in basic solvents (ZIEGLER-GÜNDER and HADORN, 1958);

sepiapteridine, SP, is a yellow pteridine described by FORREST and MITCHELL (1954), present in great quantities in the *sepia* mutant.

2-amino-4-hydroxypteridine, HB_1, is a precursor to IX (FORREST, GLASSMAN and MITCHELL, 1956; GLASSMAN and MITCHELL, 1959). The fluorescence is sky-blue and occurs in all genotypes where IX is present;

biopterin, HB_2, has been studied by VISCONTINI *et al.* (1955) and by FORREST and MITCHELL (1955). It is a very photolabile compound of general occurrence in *Drosophila*.

Isoxanthopterin was identified and well separated with both chromatographic methods used. With solvent No. 1, it had a Rf-value of 0.21 and was followed by a double spot with Rf 0.31. The latter is composed of a lighter blue compound and a yellowish one. The blue compound, judged by its color and amount in wild-type, appears to be HB_1, possibly combined with HB_2 and xanthopterin. However the latter is not known to appear after development in acid solvents. The yellow substance is probably identical with sepiapteridine. Owing to the limitations of the filter combinations of the Analytrol, the measurements of the double spot refer only to the blue compound. A visual estimation of the yellow compound revealed no great difference among the genotypes. A third very weak bluish spot with higher Rf-value was also observed after separation by method No. 1. It was neither measured, nor identified.

Using solvent No. 2, three well separated blue spots were seen. The first one was isoxanthopterin. The next was lighter blue, and probably identical with HB_1. The third spot, also light blue, was assumed to be HB_2.

The results of the quantitative estimates of these compounds are given in Tables 5—7. Males and females differ in the amounts of their fluorescent substances. Isoxanthopterin has a very high concentration in males, since it is mainly deposited in the testes. To assure reliable estimates in the Analytrol, different amounts of extract were used, depending on the amount of the substance being measured. In all comparisons between sexes the estimates were adjusted to the same amount of extract.

The amounts of the fluorescent compounds under investigation are higher in the wild-type than in the *apricot* mutant. All the reversions, however, have a higher content of fluorescent substances than wild-type. The differences are greater for males than for females, and are least for isoxanthopterin in females, which compound has the lowest concentration throughout.

Where heads and abdomens of wild-type and w^a were homogenized

TABLE 5. *Intensity estimates of isoxanthopterin, IX, measured as the integrated area in cm².*

Spot No. 1 after development in butyl ether/chloroform/acetic acid/H_2O. Homogenate: 50 intact animals per ml. Deposit: 0.04 ml for males, 0.10 ml for females.

Genotype	Males, 3 determinations		Females 1 det.
	Mean	Range	
Canton-S	7.3	7.1— 7.6	3.2
w^a	2.4	2.2— 2.7	3.0
Reversions:			
w^aR57i11	14.0	12.1—15.3	4.2
w^aR58l12	13.0	11.1—16.3	5.0
w^aRM	10.1	9.0—11.8	4.5
w^aRMc	12.8	11.3—14.3	5.8
Mean of reversions	12.5		4.9
Total mean per 0.10 ml	$9.93 \times \frac{10}{4} = 24.8$		4.3

TABLE 6. *Intensity estimates of the HB-pteridines.*

Spot No. 2 after development in butanol/acetic acid, measured as integrated area in cm². Homogenate: 50 intact animals per ml. Deposit: 0.10 ml.

Genotype	Males			Females		
	Exp. 1	Exp. 2	Mean	Exp. 1	Exp. 2	Mean
Canton-S	16.7	15.0	15.9	12.3	12.4	12.4
w^a	7.3	9.5	8.4	8.6	8.2	8.4
Reversions:						
w^aR57i11	21.5	21.0	21.3	22.1	18.8	20.5
w^aR58l12	26.5	25.9	26.2	25.4	18.9	22.2
w^aRM	21.1	21.1	21.1	15.9	14.7	15.3
w^aRMc	23.2	19.5	21.4	22.8	20.6	21.7
Mean of reversions	23.1	21.9	22.5	21.5	18.2	19.9
Total mean per 0.10 ml			19.0			16.7

TABLE 7. *Intensity estimates of 2-amino-4-hydroxypteridine and biopterin (HB_1 and HB_2).*

Spots Nos. 2 and 3 after development in butyl ether/chloroform/acetic acid/H_2O, measured as integrated areas in cm^2. Homogenate: 50 heads and abdomens for males, 50 intact females per ml. Deposit: males 0.08 ml, females 0.10 ml.

Genotype	Males						Females	
	HB_1			HB_2			HB_1	HB_2
	Heads	Abd.	Total	Heads	Abd.	Total		
Canton-S	3.8	3.3	7.1	3.2	2.0	5.2	7.4	7.7
w^a	2.8	2.5	5.3	2.0	2.1	4.1	6.0	3.2
Reversions:								
w^aR57i11	9.3	5.6	14.9	8.1	7.8	15.9	14.6	9.3
w^aR58l12	9.6	5.3	14.9	9.1	6.5	15.6	13.0	10.5
w^aRM	6.8	4.0	10.8	5.4	5.6	11.0	9.2	8.4
w^aRMc	9.2	4.2	13.4	8.2	6.0	14.2	16.0	11.7
Mean of reversion			13.5			14.2	13.2	10.0
Total mean per 0.10 ml	$11.1 \times \frac{10}{8} = 13.8$			$11.0 \times \frac{10}{8} = 13.8$			11.0	8.5

separately, and the amounts of the two compounds HB_1 and HB_2 were compared, the heads were found to have a somewhat higher concentration, the relative amounts being 1.4 : 1 for HB_1 and 1.1 : 1 for HB_2 (Table 7). Among the reversions, the three with the highest amount of pigments, w^aR57i11, w^aR58l12 and w^aRMc, have rather uniform amounts of fluorescent substances, while w^aRM, with less of eye pigments, has a comparable lower amount of fluorescent substances. The results, however, do not indicate that these substances are suitable for an exact discrimination among reversions. These analyses were undertaken at an early stage of the investigation when only four reversions were available. Since the uniformity among these was so great, those reversions available later were not studied. It was assumed that nothing significant would be revealed by such a study.

III. DISCUSSION

The main purpose of this investigation has been to find a reliable and comparatively easy method for discriminating among independent re-

verions of the mutant gene, w^a. Quantitative estimates, more sensitive than visual observation, were employed. Such methods might reveal whether the phenotypes of independent reverse mutations, randomly collected, are continuously distributed between the phenotypic limits set by the mutant and the wild-type alleles, or whether they form discontinuous groups with characteristic effects.

The results show that genuine back mutations of w^a, indistinguishable from the wild-type, have not been obtained. Apparent back mutations to wild-type have been reported for the *yellow*, *scute* and the *forked* loci in *Drosophila* (GREEN, 1957, 1959 and unpublished), but since these mutants concern morphological characters where quantitative phenotypic estimates cannot be objectively made, it is still possible that completely restored wild-types have not really been obtained. The possibility of reversion to wild-type might depend on the complexity of function blocked by the mutation, or where in the reaction chain the mutant acts. Since none of eight reverse mutations have restored the function of the wild-type allele of *apricot*, we must suppose that the chances of back mutation to wild-type of this mutant are small. This is in agreement with the observation on back mutation with *Neurospora* (GILES, 1951; GILES *et al.*, 1955).

In an attempt to obtain the most reliable measure of gene function, both pigment and UV-fluorescing compounds were analyzed for several w^a reversions. Since the fluorescent substances are known to be rather photolabile and/or difficult to sharply separate chromatographically, too much significance cannot be placed on quantitative estimates of these compounds. Consequently their utility in separating out reversions is of limited value only.

Nevertheless, comparisons among the amounts of fluorescent substances in the mutant, the reversions and wild-type reveals some important points. Excepting the drosopterins, the amounts of all the pteridines observed increase in the reversions and reach concentrations higher than those in both *apricot* and wild-type. This is in agreement with the observations made by ZIEGLER-GÜNDER and HADORN (1958), who compared $+/w$ and $+/+$ females, and found higher amounts of sepia- and HB-pteridine (jointly measured) in the heads of the heterozygous females associated with a lower amount of red pigment. The accumulation of fluorescent substances in genotypes with reduced red eye pigment indicates a relationship between these substances, even though the meaning of this relationship is not completely clear. It is clear that the interference in the normal reaction chain caused by the

apricot mutation does not affect the stage between the fluorescent substances and the visible pigment, which seem to be in equilibrium. Nor does it affect the transformation of HB_1 to isoxanthopterin, for a low amount of isoxanthopterin occurs together with a low amount of HB_1 in w^a, and a high amount of isoxanthopterin with a high amount of HB_2 in the reversions (Tables 5—7). The sepiapteridine, which in the homozygous *sepia* genotype reaches a high concentration, has not been quantitatively estimated in this investigation. It does not seem to vary much among genotypes. Presumably the transformation of sepiapteridine is also uneffected by the *apricot* mutation. It seems most probable that the *apricot* mutation alters the function of some enzymatic process early in the synthesis of the red pigments, and that the reversions only partially restore the process by means of some compensatory reaction which produces the higher amounts of fluorescent substances. This is not unlike the situation described by GILES (1956) for back mutations of the tryptophaneless-1 and adenine-3 mutants of *Neurospora crassa.*

Since the amount of red pigment never reaches the wild-type level, discrimination among reversions is best performed through quantitative estimates of this pigment. The chromatographic separation of its three component drosopterins reveals nothing exceptional. With minor discrepancies, all are affected similarly when the total amount is changed. Although the total amount of red pigment thus does not quite tell the whole story, it nevertheless gives a good indication of the nature of a reversion, and is the by far most easily and exactly obtained.

The intensities of the red and brown eye pigment are positively correlated to each other, but among the reversions this relationship does not correspond to that indicated by the mutant and the Canton-S phenotypes. If the regression lines of Fig. 1 are extrapolated to the wild-type intensity of red pigment, the corresponding amount of brown pigment is more than double that actually found in the wild-type. This aberrant relationship together with the excess of fluorescent pteridines found in the reversions indicates that the reversions are not simply hypomorphic alleles with impaired wild-type function, but represent an altered mode of action which compensates for the lack of pigment in a new way. Considering the uniformity of all of the reversions in these respects it seems likely that a real back mutation to wild-type is a rare or even impossible event.

The question of the identity of different reversions cannot yet be answered. Apparently six different types have been obtained among the eight investigated. Two reversions with low and two with high intensity

agree so closely in all the aspects studied that they seem to be identical. Whether this is a real identity can only be decided by further genetical tests using specific genes known to interact with the *apricot* genotype, such as *supressor-of-w*^{a} and *zeste*.

Acknowiedgements. — This investigation was supported by the Swedish Natural Science Research Council.

SUMMARY

Eight reverse mutations from the *w*^{a} mutant have been analyzed for their content of red and brown eye pigments and pteridines. In none has a complete restoration of the wild type gene function been obtained.

The amount of red pigment increased in all but two reversions, but never exceeded 40 % of the wild-type amount. The amount of brown pigment increased in all being completely restored in five of the reversions. One reversion contained an amount higher than the wild-type.

A positive correlation was found between the amount of red and of brown pigment, but the relationship was different from that indicated by *w*^{a} and wild-type.

The pteridines were chromatographically separated and their amounts estimated. The three component red pigments, the drosopterins, were all affected and occurred in similar relative amounts in all genotypes. Among the fluorescent pteridines, the isoxanthopterin and the HB-pterins have been estimated. All had a higher concentration in the reversions than in either the *w*^{a} mutant or in the wild-type.

It is concluded that the amounts of red and brown eye pigments give the best indication of the gene effect of the reverse mutations. It is also suggested that the divergent relationship between these pigments and the excess of fluorescent pteridines in the reversions are caused by an altered synthesis, and that the reversions do not simply represent hypomorphic wild-type genes.

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THE RELATION BETWEEN THE RATE OF INDUCED TRANSLOCATIONS AND TREATED CELL STAGES IN MALES OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

SINCE MULLER and ALTENBURG (1930) showed that translocations are obtained after irradiation of *Drosophila* males, a number of studies concerning this problem have been performed. CATSCH and RADU (1943), studying the relation between the rate of induced translocations and developmental stages of the irradiated germ cells obtained the highest frequency of induced translocations in sperms from matings during the first day after irradiation. The second mating period (2—7 days after irradiation), as well as the third period (8—13 days after irradiation), gave a lower rate, but in both mating periods about the same frequency was observed. In the fourth mating period the decrease was even more pronounced and from the 26th day after irradiation no translocation was obtained. AUERBACH (1954 a) observed the highest yield of translocations in the second brood, 5—8 days after irradiation, GLASS (1955) on the other hand obtained the maximum yield of translocations 8—11 days after irradiation, and KAUFMANN, GAY, and ROTHBERG (1949) working with chemical mutagens reported the highest frequency of translocations in spermatozoa used 13—18 days after treatment. In all the above mentioned experiments mating periods consisting of 4—6 days have been used. As already pointed out by AUERBACH (1954 b), the use of such long mating periods might obscure the sensitivity pattern. A day by day brood technique has been used by STRØMNÆS (1959) in a study concerning the mutation pattern of two wild type stocks of *Drosophila melanogaster*. He observed that in one stock the highest frequency of dominant lethals and translocations occurred on the 6th day and in the second stock on the 8th day after ir-

radiation. STRØMNÆS suggested that the difference between the stocks in the mating period yielding the highest frequency of induced translocations was due to the fact that the meiotic elimination had started earlier in one stock. Thus he concluded that there exists a difference in maturation rates between the two stocks. From the above mentioned investigations it is evident that very little is known about the relationship between the rate of induced translocations and treated cell stages. The findings are sometimes contradictory and therefore the problem has to be reinvestigated.

Since SÄVHAGEN (1959) has observed that the highest X-ray sensitivity, measured through the occurrence of XO males and non-disjunction females, corresponds to cells treated at early meiotic stages, it would be of especial interest to study the frequency of induced translocations in different mating periods. The occurrence of translocations differs from the process leading to XO males in one main point. The XO males are due to losses of paternal X- and/or Y-chromosomes that are mainly claimed to be a single hit phenomenon (MULLER, 1940) while translocations are the results of a multihit process (BELGOVSKY, 1937). In addition to this it might be supposed that translocations could be eliminated by aneuploid segregation during meiosis. Thus the present experiment was planned in order to investigate during which mating period the peak of induced translocations occurs. By using a dual-purpose stock it was possible to study in the same experiment the frequency of losses of paternal chromosomes and translocations.

That the broken ends of the chromosomes in spermatozoa do not undergo reunion before the time of fertilization was demonstrated by MULLER (1940) and confirmed by DEMPSTER (1941) and KAUFMANN (1941). OSTER (1955) showed on the other hand, that breaks induced during spermiogenesis undergo reunion before fertilization. The time of the rejoining of broken chromosome ends in spermiogenesis could be studied by dividing the dose into two fractions with an interval that could be rather well defined.

MATERIALS AND METHODS

The following stocks were used: The females were taken from a *yf*:=; *sc*^{8}*Y*; *cn bw*; *e*^{11}♀♀×*y*$^{+}$*f*$^{+}$; *sc*^{8}*Y*, *cn bw*, *e*^{11}♂♂ stock, and the males from a *y*^{16}; *cn*$^{+}$ *bw*$^{+}$; *e*$^{+}$♀♀×*y*^{16}; *sc*^{8}*Y*; *cn*$^{+}$ *bw*$^{+}$; *e*$^{+}$♂♂ stock. *sc*^{8}*Y* contains the normal allele for y-locus (*y*$^{+}$). In the experiment *yf*:=; *sc*^{8}*Y*; *cn bw*; *e*^{11}♀♀ were crossed to irradiated *y*^{16}; *sc*^{8}*Y*, *cn*$^{+}$ *bw*$^{+}$; *e*$^{+}$♂♂ (Fig. 1).

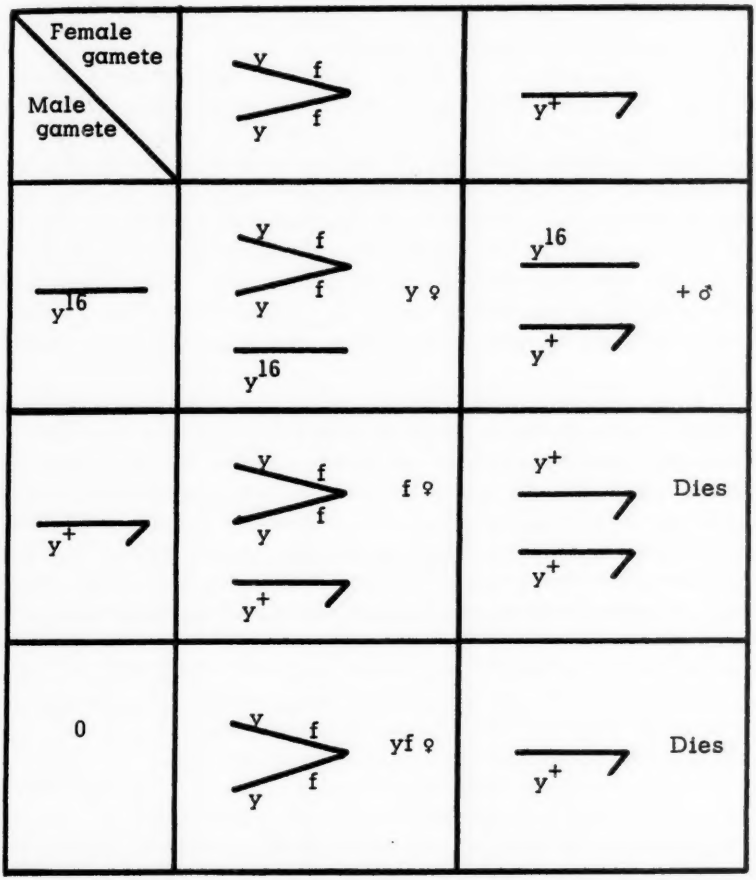

Fig. 1. The offspring from matings *y f*:=; $sc^8Y \times y^{16}$; sc^8Y. The third row shows loss of the paternal sex chromosome.

The normal offspring from this cross will be *f*♀♀ and +♂♂. There appeared also some *y*♀♀ supposedly $\widehat{XX}X$. The occurrence of *yf*♀♀ has been used as indication of genetical damage. From Fig. 1 it is seen that *yf*♀♀ result from fertilization with spermatozoa which have lost the paternal X and/or sc^8Y chromosomes. These individuals are consequently $\widehat{XX}O$ females. The XO-technique has been used by the author in another way in a study concerning the relation between the highest

X-ray sensitivity and the treated cell stages. The technique is more fully discussed in that paper (in press).

The y^{16}; sc^8Y; cn^+ bw^+; e^+ males were taken out when 0—1 day old and then irradiated when they awoke after etherization. The males were irradiated with a dose of 1100r (2 min.) by a Scandia Intensiv apparatus operated at 170 kV, 15 mA and with an inherent filtration corresponding to 2 mm Al. According to OSTER's (1955) findings that ends of chromosomes broken during spermatid stages undergo reunion before fertilization an attempt to study the effect of dose-fraction was made. The dose was divided into two equal parts (550r). Both parts were given in air atmosphere and with an interval of 1 hour. Immediately after the irradiation the males were transferred to a mating cage.

In an experiment with the present aim the irradiated males can either be mated individually to virgin females or mass-mated to virgin females for restricted periods. The latter technique is more fitted for studies concerning changes in mutation rate in successively younger germ cells at the time of treatment. The reason for this is that this method has the advantage that one will have a well defined length of time for each mating period. The time schedule for the mating periods was 0—4 hours for the first period and from the 4th day after irradiation and onwards every mating period consisted of 24 hours. Between the first mating period and the 4th day the males were kept with an excess of virgin females. At the end of each mating period the males and females were separated. The males were transferred to a new mating cage and new virgin females were introduced. The females were placed in vials, 5—7 in each. They were allowed to ovoposit for three 3-day periods. The vials were emptied on the 12th and the 15th day and the offspring were recorded. In the first generation after irradiation the frequency of induced $\widehat{XX}O$ females was scored. Among the progenies from the matings 0—4 hours, the 4th, 5th, 6th, 8th, 10th, and 14th days after irradiation males were taken out and tested individually for the occurrence of induced translocations. As the vials were emptied and the offspring recorded on the 12th and the 15th day, the F_1 heterozygous wild type males to be tested for translocations were taken both from the first and from the last scoring. This method was used in order to avoid a bias according to differences in the time needed for development between males carrying translocations and normal males. Each male was crossed to 3 virgin yf:=; sc^8Y; cn bw; e^{11} females. The F_2 offspring from this cross were examined for absence of independent as-

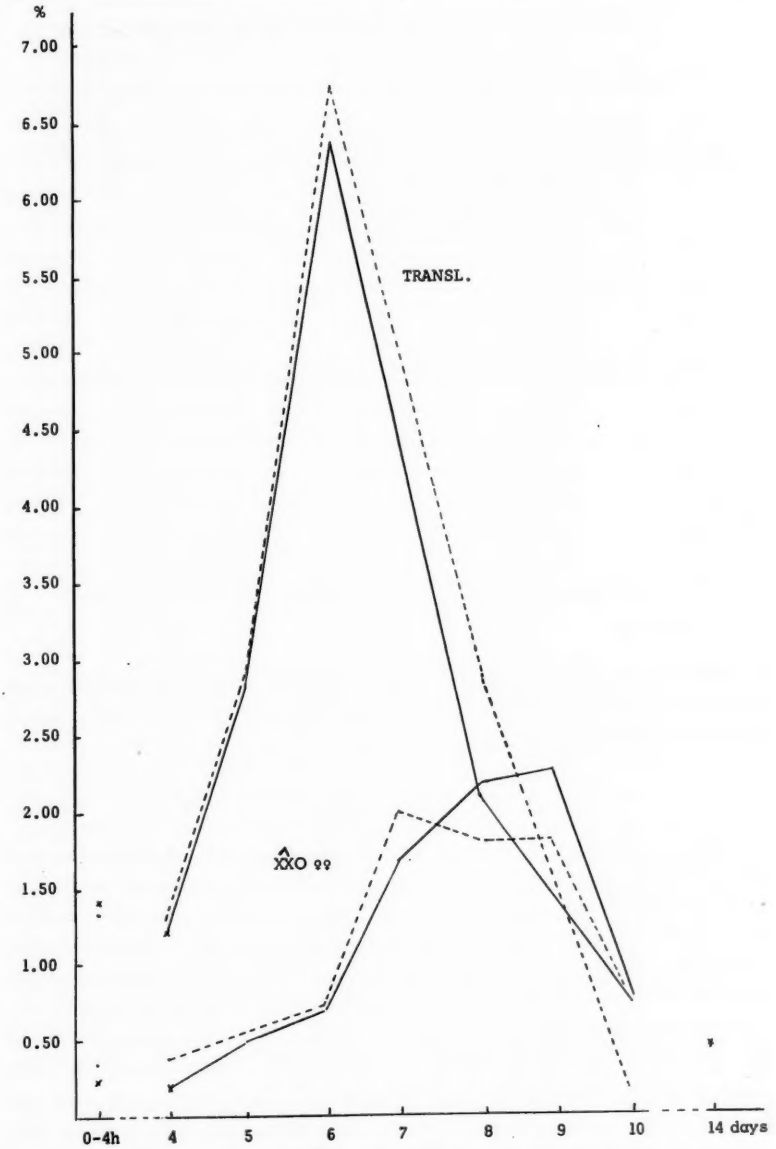

Fig. 2. The frequency of induced $\widehat{XXO}$ females and translocations in Serie A ——— and Serie B — — — —.

TABLE 1. *Losses of paternal sex chromosomes*
Serie A, 1100r. Serie B, 550r+1

	Ser.	Time from Irradiation			
		0–4 hrs	4th day	5th day	6th day
$\widehat{XX}$O females	A	26/12257 0.21 %	32/18594 0.17 %	78/15892 0.49 %	151/22480 0.67 %
	B	26/8605 0.30 %	19/5354 0.35 %	23/4428 0.52 %	37/5313 0.70 %
	Tot.	52/20862 0.25 %	51/23948 0.21 % [1]	101/20320 0.50 %	188/27793 0.68 %
	Het.	$0.30 > P > 0.20$	$0.025 > P > 0.01$	$P > 0.99$	$0.95 > P > 0.50$
Translocations	A	18/1296 1.38 %	32/2771 1.15 %	70/2507 2.79 %	255/4033 6.32 %
	B	29/2252 1.29 %	23/1806 1.27 %	33/1150 2.87 %	119/1773 6.71 %
	Tot.	47/3548 1.32 %	55/4577 1.20 %	103/3657 2.82 %	347/5806 6.44 %
	Het.	$0.95 > P > 0.90$	$0.99 > P > 0.95$	$P > 0.99$	$0.70 > P > 0.50$

[1] Heterogeneity.

sortment between *cn bw* and e^{11}. All F_2-cultures which did not yield an adequate number of flies to enable determination of whether or not a translocation was present were retested.

RESULTS

Table 1 represents the results after irradiation of 0—1 day old males with 1100r in air. The dose was delivered either as one single treatment or was divided into two equal parts (Ser. A=1100r, Ser. B=550r+1 hour interval+550r). Several experiments were undertaken for each series. The results are lumped together in Table 1. The heterogeneity between the experiments is measured through chi-square tests.

If one examines Ser. A in Table 1 and Fig. 2 one will find that in the 5th day after irradiation there is a rise in the frequency of induced $\widehat{XX}$O females. The rise is most pronounced between the 6th and 7th day after treatment. The peak is reached during the 8th and 9th day, followed by a decrease on the 10th day after irradiation. These results agree very well with a study concerning the relation between X-ray

and induction of II—III translocations.

hour interval+550r.

to mating

7th day	8th day	9th day	10th day	14th day
199/12097	168/7885	47/2123	66/8511	—
1.65 %[1]	2.13 %	2.21 %	0.78 %	—
53/2714	27/1518	30/1665	11/4102	—
1.95 %	1.78 %	1.80 %	0.27 %	—
252/14811	195/9403	77/3788	77/12613	—
1.70 %	2.07 %	2.03 %	0.61 %[1]	—
$0.30 > P > 0.20$	$0.50 > P > 0.30$	$0.50 > P > 0.30$	$0.001 > P > 0.0005$	—
—	29/1406	—	15/2009	9/1825
—	2.06 %	—	0.75 %	0.49 %
—	11/386	—	3/1515	7/1455
—	2.85 %	—	0.20 %	0.48 %
—	40/1792	—	18/3524	16/3280
—	2.23 %	—	0.51 %[1]	0.49 %
	$0.50 > P > 0.30$		$0.05 > P > 0.025$	$0.90 > P > 0.80$

sensitivity and treated cell stages in *Drosophila* males (SÄVHAGEN, in press). In that study the frequency of XO males parallels the present result with $\widehat{XX}O$ females. From the former investigation it was evident that the peak of XO males (highest sensitivity to X-irradiation) corresponds to cells treated at early meiotic stages. This conclusion was based on the occurrence of non-disjunction females. Hence in the present investigation one may conclude that cells which become available for insemination on the 7th day and onwards have been treated during meiosis (prior to anaphase I). From Table 1 it is seen that except for the 4th and 10th day after irradiation an interval of 1 hour between the two doses has no effect upon the rate of induced $\widehat{XX}O$ females (Ser. A vs Ser. B in Table 1).

Turning now to the frequency of induced translocations between the 2nd and 3rd chromosome (Table 1 and Fig. 2) we find that from the 4th day there is a significant rise in the yield of induced translocations. The peak is reached on the 6th day after irradiation. During the 8th day the rate of induced translocations is sharply reduced and on the 10th day the reduction is even more pronounced.

When comparing the effect of dividing the dose into two fractions versus a whole dose irradiation it is seen from Table 1 and Fig. 2 that an interval of 1 hour between the treatments had no effect upon the rate of induced translocations (Ser. A vs Ser. B) except for the 10th day. An interval of 1 hour was not long enough to allow rejoining of chromosome breaks giving rise to translocations.

DISCUSSION

The sensitivity pattern for induced translocations observed in the present investigation is almost consistent with AUERBACH's findings. However, instead of using a day by day brood technique AUERBACH used a brood-pattern, consisting of 4 days/brood. Thus she obtained the highest frequency of induced translocations during the 5th—8th day after treatment followed by a drop during the 9th—12th day. AUERBACH's findings were confirmed by SOBELS (1957, 1958, 1960) who studied the effect of cyanide pre- and posttreatment on the rate of X-ray induced translocations. He got the maximum yield of induced translocations in the third brood (6—7 days after irradiation), followed by a drop in the fourth brood. As an explanation for the steep decline in translocation frequency from the second to the third brood AUERBACH (1954 a) suggested that during the third brood spermatozoa had been used which were irradiated before meiosis and, when passing through the meiotic divisions, loss of translocations occurred due to meiotic segregation. The same idea has been put forward by STRØMNÆS (1959).

The opposite opinion is proposed by SCHACHT (1958) studying X-ray induction of translocations in *Drosophila melanogaster* males at different doses. He found that the majority of translocations occurred in the second and third broods (3rd—9th day after irradiation) with a maximum in the third brood (6th—9th day after irradiation) and "thus suggested in view of the relationship of the different broods to spermatogenesis that translocations were induced most readily in cells undergoing meiosis at the time of irradiation". This conclusion is astonishing since it is known that meiosis may cause elimination of translocations (ZIMMERING, 1955). However SCHACHT points out that "the assignment of stages of spermatogenesis to utilization in specific broods was a provisional one, based on the work of AUERBACH (1954) and COOPER (1950)".

From the present investigation it is evident that the brood technique

used plays an important role for the results obtained by SCHACHT. Thus it is obvious that he must have happened to include in the third brood cell stages which give the highest rate of induced translocations. When comparing the different broods with the sensitivity pattern suggested by AUERBACH, he concludes that translocations are induced in cells which were undergoing meiosis at the time of irradiation.

SCHACHT has summarized the total material for nine different X-ray doses and a control experiment (in SCHACHT, Table 3). Total sperms tested for all experiments was 1655, and 20 translocations were found. For each dose the amount of sperm tested is given. The material is very small and the brood-technique used is not satisfying, thus it is not possible to draw any safe conclusions concerning the results. This becomes even more evident when SCHACHT reports that below 1500r no translocations were recovered. To summarize the material for all four broods and give the percentage of induced translocations for each dose as SCHACHT has done is not correct, since it is evident from the present experiment that the yield of translocations varies significantly in different mating periods.

Neither does the present data agree with GLASS' (1955) observations that the obtained frequency of induced translocations in a brood corresponding to the 8th—11th day after irradiation was almost twice as great as the frequency in the first (days 1—4) and second (days 5—7) brood. This disagreement might, however, be explained on the bases of stock-differences and the brood-technique used. KAUFMANN, GAY, and ROTHBERG (1949) studying the frequency of induced translocations by nitrogen mustard in connection with near infrared radiation, observed an increase in the amount of induced translocations until the third brood (13—18 days after treatment). They were also able to check cytologically that the increase was not due to clusters of gametes carrying the same rearrangement. However they report that it appears "that exposure to nitrogen mustard may induce temporary impairment of sexual activity, but that recovery takes place in a high proportion of treated males". AUERBACH and ROBSON (1947) have suggested that mustard gas and the related substances might inhibit the progress of spermatogenesis. This retardation, and the observed impairment of sexual activity, might have caused the observed shift in sensitivity pattern for induced translocations. Besides this the brood-technique with mating periods consisting of six days might have some effect upon the results, especially if the males are not kept with an excess of females during the mating periods.

SHAPIRO and NEUHAUS (1933) studying the frequency of induced translocations in *Drosophila* males obtained the maximum yield of translocations in sperm used 6—12 days after treatment, followed by a sharp decrease during the following six days. From these results they concluded that more translocations were obtained in mature sperm than in immature. They suggested that the process giving rise to translocations was dependent on the age of the germ cells at the treatment. They supposed that during gametogenesis there exists a stage, where the chromosomes are more sticky than in other stages. During this developmental stage the chromosome fragments induced by X-ray irradiation might easily join. They also supposed that this stage corresponded to a period following meiosis. BOGATSCHENKO and LOBASHOW (1936) raised the question whether the stickiness could be the only explanation of the observed difference in the rate of translocations obtained in mature and immature germ cells. In order to gain more information they irradiated 70—75 hour old larvae and adult males with 5000r. The males from both experiments were then mated to virgin females for 5—6 days, whereupon the F_1 males were tested for the occurrence of translocations between all chromosomes except the Y chromosome. The observed lower yield of translocations in immature germ cells, obtained after irradiation of larvae, will according to BOGATSCHENKO and LOBASHOW depend upon the occurrence of reciprocal attraction forces, which are supposed to be at work between homologous loci in immature (diploid) germ cells. According to COOPER (1950), and KHISHIN (1957) the testes of 70—75 hour old larvae contain spermatogonia and spermatocytes and there is no sign of the onset of meiosis. The ideas put forward by SHAPIRO and NEUHAUS (1933) and BOGATSCHENKO and LOBASHOW (1936) are interesting but ought to be retested under more adequate experimental conditions since the sperm delivered during a period as long as 6 days are not homogenous. This might be true not only of irradiated adult males but also larvae.

OSTER (1958 a and b), studying the radiosensitivity of the male germ line for induced translocations, irradiated larvae, pupae, and adult males. When comparing the results after irradiation of these developmental stages he observed that the highest yield of induced translocations was found after treatment of the pupae. According to OSTER (1957 a) the testes in 48 hour old pupae contain spermatids. Thus he concludes that spermatids represent the most sensitive stage of the male gametogenesis to irradiation. OSTER (1957 b, 1958 a and b) suggests that the observed difference in sensitivity between spermatozoa

and spermatids might depend on the fact that more oxygen (intra- or intercellular) is present in the spermatids. This suggestion is based on studies concerning the effect of increasing the oxygen tension on the radiosensitivity of spermatids versus spermatozoa.

According to the present investigation, where only adult males have been treated and hence the different developmental stages of the germ cells are exposed to the same dose, it is obvious that the different stages in spermato- and spermiogenesis vary in the sensitivity to X-ray irradiation. It is also evident that it is not possible to speak about a maximum sensitivity to X-ray irradiation without indicating which type of damages have been studied. For example, in the present study the highest yield of induced translocations is obtained in the 6th day after treatment whereas the peak of induced $\widehat{XX}O$ females is reached on the 8th—9th day after irradiation. However, the peak in translocations observed on the 6th day after irradiation in the present investigation does not necessarily correspond to cells which are the most sensitive ones for induced translocations. The reason for this is that the recording of induced translocations is limited to viable rearrangements, and translocations induced in spermatocytes might be eliminated during meiosis. This elimination might be caused by a number of different factors, for example, unequal segregation, breaks which do not rejoin or recover, and cell lethality.

One may perhaps suppose that most translocations are eliminated during meiosis I and thus by analogy one may conclude that the observed peak of translocations correspond to cells which at the time of treatment have passed anaphase I. There is no reason to suppose that translocations which have been able to pass the first meiotic division ought to be eliminated in meiosis II if they do not cause cell-lethality. However, translocations induced in cells just passing through meiosis II may also be subjected to elimination. Thus one may suggest that the treated cells which become available for insemination on the 6th day after irradiation might chiefly correspond to early spermatid stages.

That the decline in translocation frequency obtained on the 8th day after irradiation must be due to meiotic elimination is consistent with the sensitivity pattern obtained by SÄVHAGEN (in press). The even more pronounced reduction on the 10th and 14th days after treatment might depend on elimination in meiosis as well as that the spermatozoa used during this mating period correspond to cells which at the time of treatment were less sensitive. This idea is supported by OSTER's

(1958 a) observation that very few translocations are obtained after treatment of spermatogonia.

In studies concerning radio-sensitivity comparisons are made, on the one hand, between mature spermatozoa and, on the other hand, between maturing or immature germ cells. In order to be able to make such a comparison, it seems necessary to delimit the first mating period to 0—4 hours after irradiation. The reason for this is that LÜNING (in press) has been able to demonstrate the existence of changes in the rate of induced mutations in spermatozoa released earlier than 24 hours after irradiation. However, this observation is not based on translocation studies; but NORDBACH and AUERBACH (1956), as well as ABRAHAMSON and TELFER (1956), have reported that sperm ejaculated on the second day after treatment yield a significantly lower frequency of translocations. Hence even for this type of damage some changes seem to occur during the first 48 hours after treatment.

When comparing the observed frequency of induced translocations in the first mating period (0—4 hours after irradiation) versus the 4th day, a lower frequency is obtained in the latter period. This may perhaps be due to a difference in susceptibility. Such a decline is also observed by LÜNING (1952) for dominant lethals.

CLARK (1955) studying the rate of induced 2:3 translocations in fractionated and non-fractionated experiments obtained a pattern of sensitivity for induced translocations in the non-fractioned experiment which parallels the present result. The difference presumably depends on the fact that CLARK has used mating periods consisting of three days each. In the fractionated experiment the dose of 2800r was divided into four equal parts, delivered with an interval of 24 hours between each treatment. In this experiment CLARK observed a slight decrease in the rate of induced translocations during the second brood (4—6 days after irradiation) but since the difference was not statistically significant he concluded that "whether or not there is a tendency for reunion to occur at a stage just prior to the fully matured sperm is therefore still an open question". However, except for the above discussed decrease in the second brood, the first, third and fourth brood in CLARK's fractionated experiments yielded a higher rate of induced translocations than the corresponding mating periods in the non-fractioned series. From the present investigation one must conclude that it is impossible to decide whether or not there exists a tendency for reunion at a special developmental stage when such long intervals as 24 hours are being used between the doses. During the 72 hours covered by the ad-

ministration of the fractionated dose, there must occur a successive change in the germ cells and that this change is correlated with a change in sensitivity for induced translocations is evident from the present study.

That this pattern of sensitivity for induced translocations is not something peculiar to *Drosophila melanogaster* is confirmed by studies performed on *Drosophila virilis* (ALEXANDER *et al.*, 1954, 1959; ALEXANDER, 1956, 1958; ALEXANDER and STONE, 1955; STONE *et al.*, 1954; STONE, 1956). STONE (1956), after checking the period when X-ray irradiation causes an excessive sterility, deduced by analogy, a sensitivity pattern for *Drosophila virilis* which is almost consistent with that suggested for *melanogaster* by AUERBACH (1954 b). However, *Drosophila virilis* has a longer life cycle and matures more slowly than *melanogaster,* therefore the first mature spermatozoa do not appear until the 6th day after eclosion (STONE *et al.*, 1954). After irradiation of young males STONE *et al.* observed that the highest rate of induced translocations is obtained during the 13th—15th day after treatment, which, according to the deduced sensitivity pattern, corresponds to cells treated during spermiogenesis. The peak is followed by a sharp decrease in the 16th—17th day after treatment. This period has been suggested to correspond to cells treated during meiosis. During the 18th —21st day after irradiation the rate of induced translocations is very low. It is supposed that this mating period corresponds to cells treated as spermatogonia.

In my study (SÄVHAGEN, 1959) concerning the relationship between sensitivity to X-rays and treated cell stages the peak of induced XO males coincides with the maximum yield of non-disjunction between the paternal X and Y chromosomes (non-disjunction ♀♀). Since the occurrence of non-disjunction females is a criterion of cells treated prior to anaphase I, it is concluded that the peak in the rate of XO males occurs in cells which at the time of treatment were in early meiotic stages (prior to anaphase I). In the present study the rate of induced $\widehat{XX}$O females parallels the above mentioned results with XO males, thus one may suppose that the highest yield of $\widehat{XX}$O females corresponds to cells treated in early meiosis, available for insemination 8—9 days after treatment. On the other hand, the peak of induced translocations is obtained on the 6th day after irradiation and, hence, the highest sensitivity for induced translocations might correspond to cells treated after anaphase I, presumably in early spermatid stages.

It is also evident that the rate of induced translocations varies with different cell stages in spermio- and spermatogenesis and that the highest rate is obtained on the 6th day after irradiation. Thus, even for this type of damage, the mating schedule is of importance, and, when discussing the frequency of induced translocations, this figure has to be related to a well defined mating period.

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SUMMARY

(1) The present paper deals with the relation between induced translocations and the treated cell stages in *Drosophila* males. By use of a dual-purpose stock it was possible to study the frequency of induced $\widehat{XX}O$ females in F_1 and the rate of induced translocations in F_2.

(2) The observed frequency of induced $\widehat{XX}O$ females parallels very well earlier studies with induced XO males and induced non-disjunction between the paternal X and Y chromosomes. The occurrence of non-disjunction females was used as an indication of cells treated prior to anaphase I.

(3) It was shown that the frequency of induced translocations varies with the mating periods, which, according to the brood technique, represent successively younger germ cells.

(4) The peak of translocations was observed on the 6th day after irradiation. It is suggested that the cells which become available for insemination during this mating period chiefly correspond to cells treated as early spermatids.

(5) Germ cells irradiated during early meiosis (prior to anaphase I) yield few translocations (8th day after treatment).

(6) The reduction in the rate of induced translocations is even more pronounced on the 10th or 14th day after irradiation.

(7) It is evident that there exists a difference in the observed pattern of sensitivity to irradiation when different types of aberrations are used as an indication of induced genetic damage.

(8) No effect is observed upon the rate of induced translocations when the dose is delivered in two fractions with an interval of 1 hour.

Thus an interval of one hour was not long enough to allow rejoining of chromosome breaks giving rise to translocations.

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STUDIES OF IRRADIATED MOUSE POPULATIONS

I. PLANS AND REPORT OF THE 1ST GENERATION

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INTRODUCTION

IN the studies of the mutagenic effect of ionizing radiation most experiments have been concerned with mutations which individually could be tested with appropriate methods. In recent years the interest in those mutations which could be detected only by statistical means has been more and more pronounced. This has involved questions as to the effect of radiation on populations. Most of these experiments have so far dealt with populations of *Drosophila* in which the technical facilities are most highly developed.

As it is of especial interest to get information about the hazards to which man is subjected by his increasing exposure to ionizing radiation it has seemed necessary to include as well mammalian populations in studies of the effect of radiation on populations. Most of the information about mutations induced in mice comes from the studies in the groups around Dr. W. L. RUSSELL in Oak Ridge and Dr. T. C. CARTER at Harwell on selected genes with visible effects. Professor BONNIER, 1952, worked out plans to study the effect of mutations affecting the metabolism in order to get information about mutation rates as well as the dominant effects of those mutants. These plans, however, could not be realized due to the fact that we lacked the necessary facilities at that time. In the meantime Professor BONNIER has undertaken a number of model experiments with *Drosophila* which have been of great importance in modifying the original project for the studies of mice. In the middle of 1959 a new laboratory was ready and the experiments could be started in August of that year. This paper will discuss the plan of these experiments as well as the first results obtained.

It is the purpose of these experiments to study the effect on productivity and breeding performance in a population which in each generation gets an acute dose of X-rays to the spermatogonia.

The plan

In order to get sufficient material and not too high a rate of inbreeding it was decided that the size of the population should be about 125 pairs and in any case not lower than 100. In order to be sure that irradiated spermatogonia are used the mating is postponed 90 days from irradiation. From each pair not more than 4 young are taken out to continue the population, if possible 2 females and 2 males. There are 20 cages for females and 20 for males. If the first litter consists of 2 ♀, 2 ♂ they are placed in the cages ♀ 1, ♀ 2 and ♂ 1 and ♂ 2. The young from the next litter are placed in cages ♀ 3 and ♂ 3 and so on. When one animal has been placed in each of the 20 cages with females and males respectively the process is repeated, starting again from cages ♀ 1 and ♂ 1. If 100 litters are collected there will be no more than 200 females and 200 males and probably less. Only 125 of the females and the males respectively will be used to continue the population. Hence from each box the same number of males and females is taken out. In order to secure randomness they are again divided among the new cages. When the males are 60 days old they are irradiated and after a lapse of 90 days mated to the females. By this technique the females will inherit radiated genomes from their fathers and the males will both inherit irradiated genomes and be irradiated themselves. Parallel to this there is a control population handled in the same way except for the exclusion of the irradiation procedure.

As a standard test of productivity we have chosen what we call the 100-day test. This means that each pair is allowed to be together for a maximum of 100 days or up to the time when the 3rd litter is weaned, if this occurs before the 100th day. The irradiated and the control populations are both subjected to this test. The offspring in the two populations not used to propagate the populations are subjected to the "100-day" test in such a way that there are reciprocal matings to non-irradiated animals from the inbred nucleus. The animals are mated at an age of about 60—70 days. At the outset the control-population was not simultaneous with the irradiated population but was started one month later. This has, however, been corrected so that from the 2nd generation they are simultaneous. There was an extra control population during

the first generation in order to give as much information as possible about the starting point.

The nomenclature used in the experiments requires comment. As the main interest is centred on the poststerile period the populations are called P (irradiated) and KP (control). The first generation is called P1 and KP1 and the subsequent generations P2, KP2, and so on. The extra control at the beginning was called KPO.

As daughters and sons are tested they are called:

P1X	daughters	to	P1	mated	to	males	from	$	
P1Y	sons	„	„	„	„	females	„	$	
KP1X	daughters	„	KP1	„	„	males	„	$	
KP1Y	sons	„	„	„	„	females	„	$	and so on.

The stock

All animals used in these experiments are derived from the inbred CBA. The stock was put at our disposal by Dr. CARTER, Harwell, which we very much appreciate and beg to acknowledge. They originate from one litter obtained in 1956. They have been propagated by strict brother-sister mating. During the first three years the inbred nucleus consisted of about 50 pairs. From July 1959 it consists of about 160 pairs. The relationship between pairs is not more remote than 4th cousins. The animals which constituted the populations P1, KP1 and KPO were not more remotely related than 2nd cousins.

The irradiation

The males are irradiated individually. They are taped on a plexiglas and covered by a lead shield with a hole over the testis. The apparatus is operated at 185 kVp, 15 mA, 1 Al+½ Cu. The dose rate is 69 r/min. The total dose is 276 r.

RESULTS

As the matings in Series P1 should be postponed 90 days from irradiation, the first three months were used to get training in handling the experiment and to detect fallacies in the technique. This was done by studying the effect of irradiation of mature spermatozoa. The results of these studies made it evident that the irradiation and control series had to be simultaneous. This demand was considered in the tests of the offspring to P1, KP1, and KPO (thus in P1X, KP1X, KPOX, P1Y and KP1Y). Three sets of tests were started, P1X vs KPOX, P1X vs KP1X

TABLE 1. *Breeding performance of the irradiated and two-control populations in the first generation.*

Categories	P1	%	KPO	%	KP1	%
Pairs	123		90		124	
3 litters born and weaned	53	43.1	34	37.8	66	53.2
2 „ „ „ „ 3rd born	21	17.1	8	8.9	18	14.5
3 „ „ 1—3 litters lost	36	29.3	19	21.1	20	16.1
1—2 litters born	10	8.1	26	28.9	14	11.2
Sterile	1	0.8	—	—	4	3.2
♂ or ♀ dead	2	1.6	3	3.3	2	1.6

and P1Y vs KP1Y. In each of them there was a coding by means of random tables. The cages were given a number and after mating the code was hidden until the 100-day test had been finished, when the material was decoded. By this procedure the personal bias that the animals from the two series might be handled in different ways is avoided and furthermore the environmental factors are randomized as far as possible within each pair of series.

The results of the 100-day test in the 1st generation in the three populations are shown in Tables 1—3. It ought to be emphasized that the three populations, as they were not exactly simultaneous, might have been affected by different environmental factors beyond our control. The rate of young produced is about the same in the three populations as is seen from Table 2.

Of more interest are the results in the tests of the offspring from the three populations. In this case the material was coded and the series, pairwise, simultaneous. Hence comparisons can be made on a much safer ground. The performance is presented in Table 3. From this it is evident that comparisons have to be made within the simultaneous series as there is a marked difference between the two tests of P1X.

TABLE 2. *Productivity in the first generation of the populations. For symbols, see the text.*

Series	Total	Litters	Mean litter size	Mean of 3 litters at birth
P1	2170	352	6.16	18.40
KPO	1409	231	6.10	18.69
KP1	2075	341	6.10	18.37

TABLE 3. *Breeding performance of daughters and sons in the irradiated and in the two-control populations when mated to animals from the inbred nucleus.*

Categories	P1X	KPOX	P1X	KP1X	P1Y	KP1Y
Pairs	158	158	160	160	160	160
3 litters born and weaned	32	35	68	63	77	69
2 „ „ „ „ 3rd born	12	9	11	16	16	9
3 „ „ „ „ 1—3 litters lost	97	95	66	68	55	70
1—2 litters born	11	14	10	11	6	8
Sterile	2	3	—	1	3	—
♂ or ♀ dead	4	2	5	1	3	4

The reason for this difference is partly known. During the test period a high frequency of infantile diarrhoea appeared, which was most marked in the first P1X and KPOX. Litters with diarrhoea were discarded. Hence the high rates with lost litters.

Cases in which diarrhoea had appeared were recorded, and consequently the epidemic could be of use as a stress factor possibly affecting the control and the irradiation series differentially, if the latter were in some ways inferior. In Table 4 are presented the number of pairs with 3 litters born and the occurrence of diarrhoea among them. Evidently there is no indication of a higher susceptibility among animals from the irradiated population than from the control. From Table 4 it is also obvious that the number of pairs giving 3 litters within 100 days is about the same in all series, thus indicating that with this standard it was not possible to detect any difference between the P and KP-series.

The number of young born from these pairs is presented in Table 5. The still-born are included in the number born. They are also shown separately in the table. Remarkable differences exist between the two repeats with P1X but each is in agreement with its control.

The rate of death before weaning in those cases where all three litters were weaned was also studied. These data are presented in Table 6.

TABLE 4. *The number of pairs with infantile diarrhoea among their young in the series in Table 3.*

	P1X	KPOX	P1X	KP1X	P1Y	KP1Y
3 litters born	141	139	145	147	148	148
With diarrhoea	55	49	31	31	31	37

TABLE 5. *Productivity in pairs with 3 litters born and the number of stillbirths in series from Table 3.*

Series	Total	Pairs	Mean per 3-litters	Number of stillborn
P1X	2642	141	18.74	74
KPOX	2554	139	18.37	60
P1X	2500	145	17.24	20
KP1X	2584	147	17.58	45
P1Y	2670	148	18.04	41
KP1Y	2656	148	17.95	34
X-ray	7812	434	18.00	135
Control	7794	434	17.96	139

Here it is striking that the rate of deaths in all three pairs of series is highest in the control. A heterogeneity test on the sum gives $\chi^2_c = 7.49$; $0.01 > P > 0.005$.

DISCUSSION

The results presented above are not in line with similar studies reported by RUSSELL and RUSSELL (1958). On a much larger material he found a slight decrease in the litter size at weaning after irradiation of spermatogonia compared to the controls. Furthermore he found that the productivity is lower in daughters to irradiated than to controls. No figures about this or about the technique used in these experiments are given, although these experiments are reported to be under way. Hence

TABLE 6. *Rate of death before weaning.*

Category	Born	Dead	% Dead
P1X	103	627	16.4
KPOX	129	678	19.0
P1X	116	1218	9.5
KP1X	134	1130	11.9
P1Y	129	1414	9.1
KP1Y	136	1279	10.6
X-ray	3418	3259	10.68
Control	399	3087	12.93

it is very difficult to comment upon the disagreement between our results. One point that could be of importance is the fact that in our experiments all animals are derived from an inbred stock (CBA) whereas the RUSSELL's use hybrids. That this ought to be considered is evident from some model experiments on *Drosophila melanogaster* which are under way (LÜNING and PETZÉN, unpublished). In these experiments dominant mutations causing death during larval or pupal stages have been studied. A higher rate of deaths in heterozygotes than in homozygotes was obtained. The heterozygotes in the non-irradiated series showed heterosis whereas the result was the reverse in the irradiated series. The reason for these results is not clear hitherto. The rate of hatchability of eggs in these series showed no dissimilarities that could explain the results mentioned.

SUMMARY

(1) The plan of a study concerning the effect of irradiation of spermatogonia during successive generations in a mouse population is presented.

(2) The results from the first generation have failed to show any deterioration in the irradiation series when compared with simultaneous controls.

(3) There was a lower rate of death before weaning in the irradiation series compared to the controls. This might be spurious and will be further studied in succeeding generations.

Acknowledgements. — This first report from the Laboratory of Radiation Genetics is dedicated to Professor G. BONNIER on his 70th anniversary. He was the initiator of the Laboratory, never failing to show a great interest in all planning and contributing many fruitful discussions. All his deep interest is highly appreciated and acknowledged. It is also a pleasure to acknowledge the assistance of Dr. C. RAMEL.

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THE INDUCTION OF EARLY MUTANTS IN BONUS BARLEY

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THE data presented in this paper deal with some problems pertinent to plant breeding and evolutionary theory: (1) Is it possible by means of induced mutation to increase the earliness of a crop plant, the early mutants being equal to the mother variety, in our case the Svalöf two-row barley "Bonus", as to production capacity? (2) Which mutants have the greatest practical value: those which alter only slightly the earliness as well as other properties of the mother variety, or those which alter the earliness drastically? (3) To what extent can the results in Bonus barley be extended to other varieties or even to other crop plants?

Up to 1957 twenty early mutants were isolated and tested in Bonus barley (Table 1). Time of heading was chosen as the character of selection. There is a distinct correlation between this time and the time of ripening. The latter date, however, is difficult to use for selection in the X_2 and X_3 generation. Heading time is a trait better suited for this purpose. Prior to 1956 no special arrangements were made in our irradiation experiments for a complete detection of early mutants. In the last few years, however, earliness was included in the routine analyses of the field material after artificial mutagenesis.

From several points of view Bonus barley is an excellent material for mutation research (BORG *et al.*, 1958): It can be considered a pure line; it is extremely high-yielding, has a high tillering ability and is a reasonably good malting barley. Nevertheless, it has one drawback: in certain years it shows a tendency for lodging. This is chiefly the reason why it has not become a universal variety in South Sweden. Yet, it is widely used outside Sweden. In Denmark, for instance, it is most extensively cultivated. Owing to this lodging tendency of the mother variety, early mutants of Bonus barley have no special interest in mechanized agriculture unless they have simultaneously acquired resistance to

TABLE 1. *Mode of origin of 20 early mutants in Bonus barley.*
T indicates that early 6 and 10 are associated with translocations.

Mutant	Type of treatment	Year of origin
early 1	Dry seeds, 10 000 r, X-rays	1942
early 2	KCN-soaking of seeds+10 000 r	1949
early 3	Dry seeds, 10 000 r, X-rays	1949
early 4	Dry seeds, 5 000 r, X-rays	1950
early 5	Dry seeds, 10 000 r, X-rays	1950
early 6 T	Soaked seeds, 10 000 r, X-rays	1949
early 7	Dry seeds, 5 000 r, X-rays	1950
early 8	Dry seeds, 20 000 r, X-rays	1950
early 9	Dry seeds, 10 000 r, X-rays	1953
early 10 T	Dry seeds, — 195° C, 10 000 r, X-rays	1952
early 11	Chronic gamma-irradiation during the period of heading to ripening	1953
early 12	Fast protons	1954
early 13	Dry seeds, 540 rep, neutrons	1954
early 14	Moist seeds, 12 500 r, X-rays	1953
early 15	Dry seeds, 480 rep, neutrons	1955
early 16	Dry seeds, 960 rep, neutrons	1955
early 17	Dry seeds, 960 rep, neutrons	1955
early 18	Dry seeds, 480 rep, neutrons	1955
early 19	Dry seeds, 400 rep, neutrons	1956
early 20	Dry seeds, 8 000 r, X-rays	1956

lodging. Quite remarkably this is frequently the case, partly due to the short straw of the early mutants, partly to changed internode structure, or other properties not examined here.

EMPIRICAL DATA

Earliness

As an introductory presentation of the material, heading and maturity data will be given for eleven of the twenty mutants isolated. The figures refer to the yield trials established as part of GUSTAFSSON's experiments by the Barley Department of the Swedish Seed Association at its substation, Hjerupslund, south of Lund. The analysis of Table 2 was made in 1959 by assistants of the Barley Department and it refers to the number of days elapsed from sowing to heading and ripeness, respectively.

Table 2 indicates that nine of the eleven mutants selected for early

TABLE 2. *Time elapsed from sowing to heading and ripening.*

Material	Heading time 1	Heading time 2	Ripening time 1	Ripening time 2
Bonus barley	85 days	83 days	123 days	120 days
early 1	84 „		121 „	
early 2	82 „		121 „	
early 4	84 „		121 „	
early 5	85 „		121 „	
early 6	84 „		120 „	
early 7	80 „		116 „	
early 8	78 „		114 „	
early 9	85 „		119 „	
early 10	80 „		117 „	
early 12		77 „		115 „
early 13		78 „		116 „

heading time originally showed advanced heading also in the dry year of 1959. All eleven mutants enhance maturity. If early 18, 19, 20, which have not as yet been tested in yield trials or crossing experiments, are excluded from the present discussion, the data indicate that the early mutants can be divided into two groups: one group comprising lines drastically altered as to earliness, the other comprising mutants slightly modifying the earliness. There is no distinct separation of the two groups, since some lines show a medium increase of earliness (*e.g.* early 14). In addition, earliness is greatly influenced by the climatic conditions of the year but not always in a parallel way in different varieties and mutants.

In 1959, as well as 1960, early 12 was most precocious as to heading time. The group of drastic mutants comprised in 1959 early 12, 8, 13, 7, 11, 10, in this order. The group of slightly changed mutants comprised early 2, 4, 1, 9, 6, 3, 5, in this order. The difference in heading time between early 12 and 5 covered a period of ten days. In 1959 there was a sharp distinction between the two groups of mutants. This was not the case in 1960. Here the order was early 12, 8, 11, 16, 13, 10, 7 and possibly 14 in the very early group, and early 2, 1, 4, 9, 17, 3, 5, 6, and 15 in the slightly changed group. In 1960 early 2 had a pronounced early heading, almost like that of early 14. However, all data gathered indicate that early 2 should be referred to the modifying group, early 14 to the drastic group.

Of course, in experimental yield plots as well as in large-scale prac-

tice the time of ripening is more important than the date of heading. Eleven mutants have been tested in GUSTAFSSON's experiments for periods ranging from two to nine years. A strict comparison with Bonus barley was always made. The most enhanced varieties were early 8, 7, 12 and 13. Early 12, which is generally the earliest mutant with regard to heading date, does not ripen so quickly as early 8 and 7. Early 8 ripens on an average six days sooner than the mother variety at its very place of origin (Svalöf) where Bonus barley was originally selected; and it ripens five days earlier than the widely cultivated Weibull's barley "Herta". Early 1, 4, 5 and 6 are of the same magnitude of earliness as Herta barley, *i.e.* on an average 1—1.5 days earlier than Bonus barley. Early 2 is interesting in this respect: it is decidedly earlier as to heading date than both Bonus and Herta barley and it ripens on an average between two and three days earlier than Bonus and one or two days earlier than Herta barley.

Early 6 and 10 have arisen in conjunction with translocations. It has not yet been settled by crossing experiments whether these two are linked or directly associated with a breakage point and it would be difficult to ascertain in the case of early 6, since this one acts as a modifying mutation, and the slight difference in earliness is not easily detected in hybrid offspring. In the case of early 6 the two breakage points lie in chromosome 4 (the short arm) and chromosome 7 (in the satellite). *Cf.* Fig. 1, and HAGBERG *et al.* (1960).

The number of genes responsible for the increased earliness in the slightly modifying mutations cannot now be determined. The drastic mutants fall into three or four categories. Supplemented with erectoides 16 of Maja barley (GUSTAFSSON, 1947) and an unpublished early mutant out of erectoides 23 (unpubl. data by D. VON WETTSTEIN; *ert-a*[23] in its turn originated from Bonus barley), they group themselves according to Table 3.

Crosses within the groups of *a* and *b* produce early F_1 and F_2 offspring, crossings between the mutations of groups *a*—*c*, invariably a

TABLE 3. *Categories of drastic mutants.*

Locus a	Locus b	Locus c	Locus d?
early 8	early 7	early 16	early 14
early 11	early 10 (?)		
early 12	early 13		
erectoides 16	early erectoides 23		

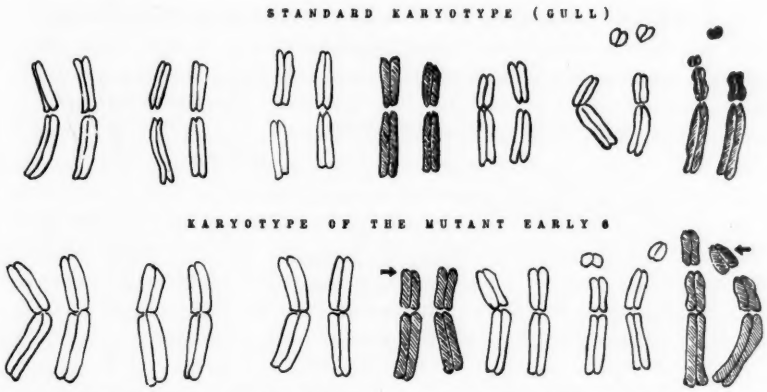

Fig. 1. The chromosomes of the modifying mutant early 6 compared with the standard karyotype. The translocation breaks in early 6 are located as indicated by the arrows in the short arm of chromosome 4 and in the satellite of chromosome 7.

late F_1, segregating for earliness in F_2. However, earliness may not be entirely recessive, since some F_1's of the mutants and the mother variety are not as late as the mother variety, as noticed long ago with regard to the F_1 hybrid of erectoides 16 and Maja barley. An interesting case of partial dominance has been noticed in early 14, which does not seem to belong to any of the three groups mentioned above. The mutant is characterized by a medium earliness, ranging between the drastic and the modifying groups. Crossed with mutants of the modifying group, it produces a late F_1 with early 1, 2, 15 and 17, an intermediary F_1 with early 3, 6 and 9, and an early F_1 with early 4 and 5. Crossed with the drastic mutants of early 11 (locus *a*), 13 (locus *b*) and 16 (locus *c*) it gives a late F_1, but an early F_1 with early 8 and 12 (locus *a*) and early 7 and 10 (locus *b*). Thus it reacts in a specific way with definite alleles of the loci *a* and *b*.

In the modifying group of mutants, early 1 and 2 apparently represent distinct loci, different from the others. Early 5 certainly gives an early F_1 with early 14, but it is probably not allelic with this mutation. Some remaining modifying mutations may be allelic (early 3, 4, 9, 15). A definite judgment must be postponed until a more detailed crossing analysis with several replications has been carried out.

Suffice it to state that at least three or four gene loci cover the drastic earliness mutations so far analysed, and that a couple of loci have been found for the modifying mutations. Since the property of "slightly en-

TABLE 4. *Lodging of the various mutants, as compared with Bonus barley.*

Variety	Years tested	Average deviation from Bonus	
		up to 1959	in 1960 [1]
Modifying mutants:			
early 1	1951—1959	+ 0.73	—
early 2	1953—1959, 1960	+ 0.77	+ 2.33
early 4	1955—1959, 1960	− 0.55	±0.00
early 5	1955—1959	− 0.25	—
early 6	1958—1959	+ 0.13	—
early 9	1958—1959, 1960	− 0.12	− 1.00
Drastic mutants:			
early 7 (*ea-b*^{7})	1955—1959, 1960	+ 0.13	+ 2.33
early 8 (*ea-a*^{8})	1955—1959, 1960	+ 0.70	+ 4.00
early 10 (*ea-b*^{10})	1957—1959, 1960	− 0.18	+ 1.00
early 11 (*ea-a*^{11})	1960	—	+ 2.66
early 12 (*ea-a*^{12})	1958—1959, 1960	+ 0.25	+ 3.00
early 13 (*ea-b*^{13})	1958—1959	− 0.35	—
early 16 (*ea-c*^{16})	1960	—	+ 3.66

[1] The amount of lodging in 1960 was determined by GUSTAFSSON ten days before maturity.

hanced earliness" is rather complex in character and depends on many morphological, including anatomical, and physiological changes, we may expect a fairly large number of independent early mutations to appear, representing numerous gene loci.

Lodging resistance

Lodging in barley varies greatly from year to year. Primarily it depends on an interaction between the variety and the amount of precipitation during the growing season, especially at a late stage, but the type of soil in combination with fertilization and precipitation also exerts a considerable influence. At the substation of Hjerupslund the year 1959 was extreme with almost no lodging of Bonus barley, its mutants or the control varieties, whereas the year 1958, like 1960, was characterized by a severe lodging of most varieties and mutants. A comparison with Bonus barley is presented in Table 4, where the minus sign indicates a decrease, the plus sign an increase of resistance (lodging scale 1—10).

Most mutants tested for a period of years are rather similar to Bonus barley with respect to lodging properties. One drastic mutant and two modifying mutants (early 8, 1 and 2) are most definitely more resistant. Possibly this is also the case with early 11 and 16. Early 4 and 9 seem to be inferior. It is of considerable interest that mutations selected for earliness also change other properties of agricultural value. As will soon be indicated, this is probably connected with definite characters of the straw which after mutation cause the enhanced earliness. One of the mutants tested, early 8, surpasses most commonly cultivated Swedish varieties as to lodging resistance (p. 686). Mutants of locus *b* equal Bonus barley in this respect, whereas, in addition to early 8, also early 11 and 12 of locus *a* appear to be superior, like the single mutant found of locus *c*.

Lodging resistance is affected by a great morphological and anatomical properties, for instance, total straw (and culm) length, number and length of internodes (v. WETTSTEIN, 1954; EHRENBERG *et al.*, 1956; v. WETTSTEIN *et al.*, 1959), cross-section area of the stem (v. WETTSTEIN *l.c.*), sclerenchym formation in various parts of the stem, type of root system, and yield capacity of the mutants.

Nine of the mutants discussed in this treatise have a total straw length equalling that of Bonus barley: early 1, 2, 3, 4 of the modifying group, 7, 10, 11, 12, 14 of the drastic group (measurements made in 1960 are not obtained from yield trials, but from plots of the "mutation museum"). Definitely longer straw characterizes early 5, 6 and 9 of the modifying group. These also have a longer culm. Definitely shorter straw is found in early 15 of the modifying group and in early 8, 13 and 16 of the drastic group. Almost every year early 7, too, has a shorter straw. The mutants mentioned have a reduced culm length. Generally the spike length is directly proportional to the total straw length, but early 4 of the modifying group and early 10, 11, 12 and 16 of the drastic group have proportionally a reduced spike, early 14 a longer one. In all the other cases, the mother variety and the mutants have equal relative lengths of the spikes, amounting to approximately 15 per cent of the total straw length (measured in the tallest tiller of a plant individual).

It was shown in the paper of EHRENBERG *et al.* (1956) that the early mutants also have a changed internode number. With regard to this property (Table 5) early 4 and 6 of the modifying group and early 14 of the drastic one are unchanged. Early 9, 2, 3, 15, 1 and 5 of the modifying mutants seem to reduce the internode number slightly, whereas

TABLE 5. *Internode number of early mutants in Bonus barley (1960).*

Material	Intermodes per culm				Average no. of internodes	No. of tillers and plants measured
	4	5	6	7		
Bonus 1	—	2	17	1	5.95	20
„ 2	—	2	17	1	5.95	20
Modifying mutants:						
early 1	—	6	13	1	5.75	20
early 2	—	4	16	—	5.80	20
early 3	—	5	14	1	5.80	20
early 4	—	2	16	2	6.00	20
early 5	—	7	13	—	5.65	20
early 6	—	2	16	2	6.00	20
early 9	—	4	15	1	5.85	20
early 15	—	4	16	—	5.80	20
Drastic mutants:						
early 7 (*ea-b*^{7})	—	6	9	—	5.60	15
early 8 (*ea-a*^{8})	1	19	—	—	4.95	20
early 10 (*ea-b*^{10})	—	12	8	—	5.40	20
early 11 (*ea-a*^{11})	—	14	6	—	5.30	20
early 12 (*ea-a*^{12})	—	16	4	—	5.20	20
early 13 (*ea-b*^{13})	—	19	1	—	5.05	20
early 14 (*ea-d*^{14}?)	—	1	17	2	6.05	20
early 16 (*ea-c*^{16})	—	12	8	—	5.40	20

among the drastic mutants the decrease becomes progressively more distinct from early 7, 10 and 16, to early 11 and 12 and finally to early 13 and 8. The last-mentioned mutants have approximately one internode less per culm than Bonus barley itself. The average number of internodes per culm is for Bonus barley 5.95, for the eight modifying mutants 5.83 and for the drastic mutants 5.36: a slight tendency in the former group of mutants, a marked decrease in the latter group.

As mentioned previously, lodging resistance is correlated not only with internode number but also with internode length. Thus it is interesting to measure the upper first internode and the basal internodes as to their respective lengths in relation to the culm length. This is done in Table 6.

Evidently there is an increase in length of the upper first internode in early 9, 15, 5 and 6 of group 1 and in early 12, 16, 8, 11 and 14 of group 2. With regard to the basal internodes almost all early mutants,

TABLE 6. *Internode lengths in early mutants of Bonus barley (1960).*

Material	Length of internode 1 (per cent of culm length)	Length of internodes 5-7 (per cent of culm length)	Internode index 1/5-7	No. of plants
Bonus 1	29.0	16.0	1.81	20
„ 2	33.0	14.8	2.23	20
Modifying mutants:				
early 1	31.2	12.2	2.56	20
early 2	29.0	10.4	2.79	20
early 3	29.6	14.5	2.04	20
early 4	32.9	12.9	2.55	20
early 5	34.9	11.4	3.06	20
early 6	34.1	11.9	2.86	20
early 9	37.5	12.7	2.95	20
early 15	36.4	11.3	3.22	20
Drastic mutants:				
early 7 (*ea-b*^{7})	33.1	9.9	3.34	15
early 8 (*ea-a*^{8})	36.2	5.2	6.96	20
early 10 (*ea-b*^{10})	31.9	8.7	3.67	20
early 11 (*ea-a*^{11})	34.5	7.2	4.79	20
early 12 (*ea-a*^{12})	38.2	6.3	6.06	20
early 13 (*ea-b*^{13})	33.7	6.9	4.88	20
early 14 (*ea-d*^{14}?)	34.0	13.9	2.45	20
early 16 (*ea-c*^{16})	36.7	6.7	5.48	20

except the modifying early 3 and the medium drastic early 14, decrease their internode length in relation to the culm length.

Summarizing the differences between the early mutants and the mother variety as to the straw properties examined, we get the following sequences:

I. *Total straw length* (>10 per cent plus or minus deviations from Bonus average): Early 9>5, 6, 3, Bonus$_1$, early 1, *10, 11, 14*, Bonus$_2$, early 7, 4, *12*, 2, >15>*16*>*13*>*8*.

II. *Spike length* in percentage of total straw length (>10 per cent plus or minus deviations): Early *14*>1, Bonus$_2$, early 15, Bonus$_1$, early 6, 9, 2, 3, *8*, 5, 4, 7>*13*>*12*>*11*>*10*>*16*.

III. *Internode number* (>10 per cent minus deviations): Early *14*, 6, 4, Bonus$_1$ and Bonus$_2$, early 9, 2, 3, 15, 1, 5, 7, *10, 16*>*11*>*12*>>*13*>*8*.

IV. *Relative length of first upper internode* (>10 per cent plus devia-

tions): early *12*>9>*16*>15>*8*>5>*11*>6, *14*, *13*, 7, Bonus$_2$, early 4, *10*, 1, 3, 2, Bonus$_1$.

V. *Relative length of basal internodes* (>10 per cent minus deviations): Bonus$_1$, Bonus$_2$, early 3, *14*>4>9>1>6>5>15>2>>7>*10*>*11*>*13*>*16*>*12*>8.

VI. *Internode index, i.e. the relationship between upper and basal internodes* (>20 per cent plus deviations): Early *8*>*12*>*16*>*13*>>*11*>*10*>7>15>5>9>6>2>4>*14*>Bonus$_2$, early 3, Bonus$_1$.

VII. *Lodging resistance* in yield trials: Up to and including 1959 — less than that of

Bonus: early 4.

±equal: early 5, 6, 9, 7, *10*, *12*, *13*.

higher: early 1, 2, *8*.

In 1960: early *8*>*16*>*12*>*11*>2, 7>*10*>Bonus, early 4>9.

This analysis shows that early mutants often decrease their straw length and internode number, and change the proportions of the individual internodes, especially the basal ones. Simultaneously, some mutants both in the modifying and in the drastic group, increase their lodging resistance.

Finally, it ought to be emphasized that with regard to the internode index, apparently a highly characteristic feature, the modifying mutants show a slight increase. The internode index of the exceptional mutant early 14 of the drastic group is unchanged or slightly increased. The indices of the other drastic types are largely changed, and the locus-*a* mutants (8, 11, 12) have indices higher than those of the locus-*b* mutants (7, 10, 13). Also, there seem to exist differences in this respect between allelic mutations within a locus.

In 1956 an analysis was performed by crossing the drastic mutants early 7 and 8 with the modifying mutants early 1, 3, 4, 5 and 6. The low internode indices dominated in the F_1.

Production capacity

Every mutant isolated by GUSTAFSSON, LUNDQVIST and co-workers is scrutinized in small hand-sown plots before large-scale testing is carried out by the Seed Association. If a mutant looks attractive, it will be propagated and included in field trials. To avoid subjective judgments, the trials are laid out, harvested and analysed by the Barley Department. Three or four replications are generally used, with 200, 400 and pos-

TABLE 7. *Yield of the mutants of the modifying group.*

Material	No. of years tested	Average yield, kg/hectare	Mutant yield if Bonus = 100	Mutant yield if Herta = 100
Svalöf				
early 1	1951—1955	5 181	97.4	103.6 [1]
early 2	1953—1955	5 221	95.9	101.4
early 4	1955	4 908	100.5	100.8
early 5	1955	4 997	102.5	102.7
early 6	1955	5 118	107.0	107.2
Mean		5 085	100.7	103.2
Hjerupslund				
early 1	1956—1959	6 454	97.1	103.0
early 2	1956—1959	6 647	100.0	106.1
early 4	1956—1959	6 290	94.6	100.4
early 5	1956—1959	6 691	100.7	106.8
early 6	1956—1959	6 669	100.4	104.5
early 9	1958—1959	5 816	92.8	96.6
Mean		6.428	97.6	102.9

[1] 1952—1955.

sibly 600 kilograms of nitrate fertilizer per hectare, in addition to the basic phosphorus and potassium dressing. Prior to 1955 the field trials were performed at Svalöf, from 1956 at the substation Hjerupslund, south of Lund.

Dealing first with six mutants of the modifying group (early 1, 2, 4, 5, 6, 9) the yield data at Svalöf and Hjerupslund are recorded in Table 7.

The data seem to indicate that the six modifying mutants tested are equal or slightly inferior to the mother variety in respect of yield. If early 9, which is definitely inferior, is excluded, the agreement becomes even closer. Simultaneously, the mutants increase earliness by one, two or three days and some (early 1 and 2) also increase the lodging resistance. The six modifying mutants definitely surpass Weibull's Herta barley with regard to yield capacity; they have equal degree of earliness but are less resistant to lodging than this variety.

On a scale larger than that applied for the other modifying mutants, early 2 has been tested not only in Skåne but also in the provinces of Östergötland, Värmland and Uppland. The yield data are given in Table 8.

Fig. 2. Svalöf's Bonus barley (to the left) and its modifying mutant early 2 at the time of heading.

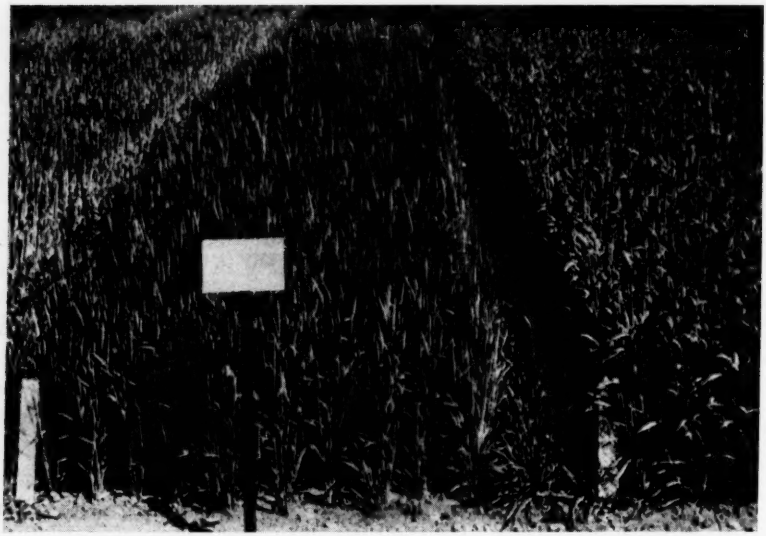

Fig. 3. Early 8 (Svalöf 04080), a drastic mutant, *ea-a*^{8} (in the middle), compared with Weibull's Rika barley (to the extreme left) and early 9, a modifying mutant (to the right), at the time of heading.

Fig. 4. Weibull's Ingrid barley (to the left) and the drastic mutant early 12, *ea-a*^{12}, at the time of heading.

Fig. 5. The drastic mutant early 8 (Svalöf 04080) compared with the modifying mutant early 2 (to the right) shortly before ripening. Note the pronounced differences as to earliness and lodging resistance.

TABLE 8. *Yield of the early 2 mutant.*

No. of trials	Yield kg/hectare	Bonus = 100	Herta = 100	Rika = 100
13	5 824	98.6	—	—
15	6 025	—	105.5	—
7	5 115	—	—	104.5

In the yield trials, early 2 equals Bonus barley and exceeds the well-known Weibull varieties Herta and Rika. The importance of this apparent superiority should not be overemphasized. Yet, it demonstrates the high-ranking productivity of the mutant. Moreover, in these experiments the mutant ripened three days before Bonus and Herta and one day before Rika.

Five drastic mutants (early 7, 8, 10, 12 and 13) have been tested at Svalöf and Hjerupslund in GUSTAFSSON's experiments; early 7 and 8 one year at Svalöf (1955), and all of them in the following years at Hjerupslund (Table 9).

On an average, the five drastic mutants are 8 per cent inferior to their mother variety at Svalöf and Hjerupslund, and early 7, 8 and 10 3 per cent inferior to Herta. Owing to its considerable precocity, high lodging resistance and fairly high yielding performance, early 8 immediately attracted a good deal of interest, also from a practical point of

TABLE 9. *Yield of drastic mutants.*

Material	Years tested	Average mutant yield, kg/hectare	Mutant yield if Bonus = 100	Mutant yield if Herta = 100
Svalöf				
early 7 (*ea-b*^{7})	1955	4 658	95.5	95.7
early 8 (*ea-a*^{8})	1955	4 795	98.3	98.6
Hjerupslund				
early 7 (*ea-b*^{7})	1956—1959	6 224	94.1	98.1
early 8 (*ea-a*^{8})	1956—1959	5 905	89.3	93.8
early 10 (*ea-b*^{10})	1957—1959	6 003	96.6	99.6
early 12 (*ea-a*^{12})	1958—1959	5 495	84.8	—
early 13 (*ea-b*^{13})	1958—1959	5 586	86.1	—
Mean: 7, 8, 10, 12, 13:		5 773	91.8	—
„ : 7, 8, 10:		5 844	—	97.0

TABLE 10. *Early 8 (=Sv. 04080) in yield tests of the Swedish Seed Association (1955—1959).*

Location	No. of tests	Yield of Sv. 04080 kg/hectare	Mutant yield if				
			Herta =100	Ymer =100	Rika =100	Edda I =100	Edda II =100
South Sweden	12	5 528	96.5	—	—	—	—
„ „	16	5 432	—	91.9	—	—	—
„ „	8	4 508	—	—	94.9	—	—
„ „	4	4 011	—	—	—	—	114.0
Middle Sweden	3	4 618	148.1	—	—	—	—
„ „	10	3 523	—	100.0	—	—	—
„ „	6	4 283	—	—	83.2	—	—
„ „	8	3 614	—	—	—	—	109.4
North Sweden	7	4 034	—	—	—	96.0	—

view. Assigned the Svalöf register number "Sv 04080", it has been extensively tested in the yield trials of the Seed Association in various parts of Sweden. In 1957 it was included in the official yield tests of the Swedish State Agricultural Research Institute. Soon it was found to maintain its relative earliness in the whole of Sweden, being characterized as a new type of two-row strain in the Swedish barley assortment. Currently propagated on a large scale, it has been suggested for release as an early trade variety of two-row barley. This, however, is not yet finally decided. Certainly it is remarkable that a mutant, arisen from a South-Swedish variety, can perform so well over large parts of Sweden, even in very northerly provinces. Table 10 records yield data obtained from the experiments of the Seed Association (including those of GUSTAFSSON).

Three South-Swedish two-row varieties and two North-Swedish six-row varieties are included in the comparison. Early 8=Svalöf 04080 is decidedly superior to the North-Swedish Edda II in South and Middle Sweden and slightly inferior to Edda I in North Sweden. It is inferior to the two-row varieties in South Sweden by some 5 per cent, but it is approximately equal in Middle Sweden. In North Sweden, where two-row varieties are not grown easily, it out-yielded the single variety simultaneously tested (Svalöf's Freja barley, not included in Table 10) by some 10 or 12 per cent.

The official tests of the State Agricultural Research Institute include a large number of varieties (Table 11). Three two-row varieties, Ymer, Rika and Ingrid barleys, are considered in the data reproduced below

TABLE 11. *Early 8 (=Sv. 04080) in yield tests of the State Agricultural Research Institute (1957—1959).*

Location	No. of tests	Yield of Sv. 04080 kg/hectare	Mutant yield if				
			Ymer =100	Rika =100	Ingrid =100	Edda II =100	Åsa =100
South Sweden	12	3 150	92.5				
„ „	12	3 150		89.7			
„ „	12	3 150			86.9		
„ „	14	2 779				106.5	
„ „	7	3 514					121.8
Middle Sweden	35	2 469	95.8				
„ „	59	2 259		97.3			
„ „	51	2 268			94.0		
„ „	35	2 319				101.8	
„ „	7	3 046					130.7
North Sweden	8	2 305		107.2[1]			
„ „	14	2 144			99.8[1]		
„ „	27	2 234				108.2	
„ „	4	2 493					104.9

[1] In the southernmost administrative province of North Sweden. Farther north two-row varieties were not included in the official yield tests.

as well as two six-row varieties Edda II and Åsa, both of North-Swedish origin.

In South Sweden, Svalöf 04080 is inferior in production by some 10 per cent. In Middle Sweden this inferiority is less marked (circa 4 per cent), and in North Sweden, Svalöf 04080 appears equal or even superior to the varieties Rika and Ingrid. In all regions where comparable tests were performed, it was superior to the early six-row varieties Edda II and Åsa.

In 1956—1959 the Barley Department of the Seed Association made a careful comparison between Svalöf 04080 and a number of other varieties with respect to lodging properties and earliness. The results agree with the data previously reported (Table 12). Here Svalöf 04080 has been compared with seven trade varieties of two-row barley. It was invariably superior to all of them in lodging resistance, even to the very stiff varieties of Herta, Rika and Ingrid. It also definitely exceeded the two six-rowed varieties tested. With regard to earliness, Svalöf 04080 ripens five—six days sooner than the two-row trade varieties, with the exception of Freja barley, which is only slightly later. It is somewhat earlier than Asplund barley but later than Edda II.

TABLE 12. *Lodging resistance and earliness of "Svalöf 04080" (early 8) in comparison with various trade varieties (trials of the Seed Association, 1956—1959).*

No. of yield trials	Sv. 04080	Bonus	Herta	Ymer	Rika	Pallas	Ingrid	Freja	Asp-lund	Edda II
Lodging resistance (1—10)										
12	7.9	6.0								
25	7.4		7.2							
34	8.1			6.7						
38	8.1				7.8					
31	7.9					6.9				
17	7.3						7.0			
14	8.9							7.8		
5	9.3								6.5	
10	9.7									8.6
Earliness (days to maturity)										
11	106	112								
23	106		111							
30	103			109						
35	104				110					
25	103					109				
15	103						108			
11	100							101		
5	93								94	
7	96									89

Other characteristics of the early mutants

Seed samples of the mutants were regularly examined with respect to 1,000-grain weight, hectolitre weight and the proportions of different seed categories after sifting. Early 4 alone clearly deviated with regard to its 1,000-grain weight, which was distinctly higher than that of Bonus barley, both in the case of light and heavy nitrogen dressings. All the other mutants approximated the value of Bonus in this respect, with small variations from one year to another. Characterized by a reciprocal translocation, early 6, too, possibly showed some increase, although slight. The increase of kernel weight in early 4 is reflected in the sifting data. This mutant produced values superior to those of the Bonus variety itself and the other mutants; after sifting (sieve size

2.8 mm), more seed gathered in the fraction of large seeds and less seed in the smallest fraction ("seed rests"). No striking differences were encountered with regard to hectolitre weight, all mutants tested approximating the figures characteristic of the mother variety.

DISCUSSION AND CONCLUSIONS

Sixteen of the twenty early mutants selected in Bonus barley have been more or less carefully examined with regard to various morphological and physiological properties, including earliness, lodging resistance, internode structure and grain production. Most mutants were included in yield trials and owing to its prominent features one mutant (early 8) obtained a Svalöf register number (Svalöf 04080). This mutant is included in the official testing and is propagated on a large scale.

The mutants represent two groups: one group slightly modifying earliness (one or two days of enhanced maturity), the other group drastically altering it (up to one week). The slightly modifying mutants produce nearly as much grain in yield trials as Bonus barley itself. Early 2 is as high-yielding as the mother variety, and is definitely earlier and decidedly stiffer in straw. Owing to the occasionally heavy lodging of Bonus barley, compared with trade varieties such as Weibull's Herta, Rika, Ingrid and Svalöf's Pallas (*v.* p. 691), the modifying mutants of Bonus barley cannot profitably be released as new varieties, although one or two of them certainly are superior to the mother strain with regard to lodging resistance. Two mutants (early 6 and 10) are connected with reciprocal translocations. In early 6 the breakage points are located in the short arm of chromosome 4 and in the satellite region of chromosome 7. The morphological and physiological properties of early 6 are largely unchanged.

The drastic mutants represent changes in three, possibly four, different gene loci (*a*—*d*). The mutants tested rate, on an average, 8 per cent below Bonus barley with respect to yield. Mutants of locus *b* yield more than those of locus *a*. Mutants of locus *a*, on the other hand, are generally more resistant to lodging than the locus-*b* mutants.

Parallel to the enhancement of earliness, whether the slightly or the drastically changed type, there are definite changes in straw structure. In comparison with Bonus barley the mutants show one or more of the following properties that influence lodging resistance (p. 683): reduced straw (and culm) length, lower internode number, longer first inter-

node, shorter basal internodes, high "internode indices". Thus enhanced earliness is automatically accompanied by such changes, which in their turn lead to an increase of lodging resistance. There is no definite correlation, since other morphological and physiological properties as well are involved in the realization of resistance. However, the most resistant mutants always have short basal internodes and high internode indices. The stiffest mutant (early 8=Svalöf 04080) has the shortest straw, the lowest internode number, the shortest basal internodes and the highest internode index of all mutants tested. The mutant is also characterized by an altered root system which develops very quickly after seed germination and penetrates deeply into the soil (ROY, unpubl.); consequently it utilizes and profits by a high water supply in the first period of growth and it is relatively less affected by summer drought than other mutants and varieties. The good yield after the severe summer drought in Middle Sweden in 1959 illustrates this feature well. In 1960, when the subsoil water was low in spring and early summer, it will probably yield less than the standard varieties.

Early 8 attracted a good deal of interest and it was soon included in the land-wide yield trials arranged by the Swedish Seed Association and later by the State Agricultural Research Institute. Although definitely inferior in grain yield to Bonus barley and other wide-spread varieties in South Sweden by 5—10 per cent, it equals the two-row varieties tested in Middle Sweden. In North Sweden it surpasses them or even grows where two-row trade varieties cannot profitably be cultivated. Its earliness, relative to that of other varieties, remains unchanged throughout its range of cultivation. Moreover, it is definitely more resistant to lodging than any trade variety tested.

Early 8 is thus a further example of a drastic mutation, which has obtained a definite agricultural significance. It is yet not ascertained whether it will be released as a new trade variety. Even in the very fertile districts of South Sweden there is a great need of early barley varieties, partly in order to spread harvesting over a longer period of time than is now possible. In Middle Sweden early maturity is definitely of special importance, since it will guarantee a good ripening of the grain also in summers with inferior harvesting conditions.

Pallas barley exemplifies a drastic mutation (erectoides 32 in locus *k*). It was released without further breeding work as a trade variety, with a resistance to lodging considerably superior to that of Bonus barley (BORG *et al.*, 1958; BORG, 1959). Moreover, it behaves like a "nitrogen ecotype", since it profits from high nitrogen dressings relatively more

than other varieties (GUSTAFSSON, 1954; BORG *et al.*, *l.c.*). Likewise, early 8 (Svalöf 04080), arisen by a one-step mutation, can be considered a "clima ecotype" of Bonus barley, with considerably increased lodging resistance in addition to its enhanced earliness. With regard to production capacity it equals the high-bred two-row varieties in Middle Sweden and surpasses them farther north, where two-row varieties are not easily grown. But even in South Sweden its earliness and lodging resistance are so valuable that the concomitant decrease in grain production may be tolerated under certain conditions and its use in practice be justified also there.

The model experiments reported here definitely show that drastic mutants may obtain an immediate practical value. The experiments also indicate that the variety used as initial material is largely decisive. Mutants of Bonus barley that modify earliness are worthless, since the mother variety is comparatively susceptible to lodging and no modifying mutants show the profound increase in resistance required. However, in varieties which fulfil the basic requirement of high lodging resistance, modifying mutations that enhance earliness and simultaneously increase lodging resistance may become of immediate practical value. Thus, the assumption that *only* drastic mutations, or *only* modifying mutations, are of practical (or evolutionary) significance, must be considered erroneous. In certain circumstances, depending on the properties of the mother variety used and the specific changes desired, drastic mutations may be needed. In other conditions, depending on the variety used and the character required, modifying mutations may be successful. Therefore, in mutation breeding one has to select the mother strain most suitable for experimentation and choose the properties which can easily be improved, *either* by drastic *or* by modifying mutations (or by both). The induction of valuable mutations is not haphazard: it is, on the contrary, a method of plant breeding requiring careful planning with a clear statement of the aim of the breeding work, a suitable mutagenic agent, and adequate selection methods.

This special point can be further illustrated by the erectoides mutations in barley (GUSTAFSSON, 1954; BORG *et al.*, 1958). Since the varieties Gull (Golden), Maja and Bonus are fairly lax-eared, their use in the selection of straw-stiff erectoides mutations, which increase ear density, will lead to less drastic and less damaging effects than those obtained with dense-eared varieties. In varieties like the new Svalöf cross variety 02102, a malting barley more dense-eared than Bonus barley, erectoid mutations will probably less frequently become of practical

value, since the ears of the mutants will tend to be too dense and the culms too short. This is even more clearly the case when the erectoid mutations are used themselves as material for the induction of "valuable" erectoid mutations. Unpublished data by Dr. D. VON WETTSTEIN show that such mutations out of erectoides 23 (=Svalöf 04023, *ert-a*^{23}) are practically useless, since the double-recessives induced often give a conspicuous "*zeocriton* appearance" with very short and even deformed ears. Also the extremely early mutation, obtained from this mutant and located in early-locus *b* (p. 678), is worthless, since the straw is too short and the production capacity is impaired.

Yet, in varieties like the ones mentioned above, Svalöf 02102 or 04023, with good lodging resistance, high productivity and other high-ranking qualities, mutations that slightly improve earliness, lodging resistance or even productivity, would be the ones required still further to improve the varieties. Such modifying mutations are now selected in experiments with Svalöf 02102. Some highly interesting cases of advanced earliness or further increased lodging resistance have also been found by VON WETTSTEIN in the mutant Svalöf 04023.

Here it ought to be emphasized that the best and highest yielding erectoides mutant of Golden barley was erectoides 1 in locus *c*, that of Maja barley erectoides 12 in locus *l*, and that of Bonus barley, finally, erectoides 32 in locus *k*. Against the background of specific genotypes mutations of different loci will apparently reach the highest production capacity and attain practical significance. Likewise, with regard to drastic earliness, locus-*a* mutants will be especially satisfactory in high-yielding but lodging-susceptible varieties, whereas locus-*b* mutants will be worth inducing in highly lodging-resistant but less-yielding varieties. This result further calls for a carefully planned mutation work by means of closely observed mother varieties with clearly stated properties. Furthermore, the breeder must learn to know the mutation spectrum and the locus behaviour of the species and variety concerned.

Induced allelic mutations of one and the same locus often — or always — are different, not only in the property specially studied, *e.g.* the erectoid character or the earliness, but also in other features pleiotropically influenced by the mutation. This was pointed out for the erectoides mutations by HAGBERG (1959), and it has been specially studied by PERSSON (unpubl.). It is also valid for the mutations that lead to drastic earliness (locus *a* and *b*). Induced mutations often influence not only the subloci within the locus but also neighbouring loci. HAGBERG (*l.c.*, p. 239) has briefly discussed such an instance with regard

to the *ert*-locus *m*. A similar case is probably provided by the mutation erectoides 16 and early 8, which are allelic with regard to earliness. No other mutations of locus *a* have the characteristic properties of erectoides 16, its intense anthocyanin pigmentation of the protruding awns and its yellowish discolouring of the sterile side flowers shortly before maturity, although several mutants of the *a*-locus show an increased lodging resistance in consequence of their new internode structure.

To what extent are the results obtained with regard to earliness in Bonus barley valid also for other strains and species? In species like barley, diploid wheat and the snapdragon early mutants are rather common. A specially drastic mutant with low productivity was recorded by SMITH (1939) in *Triticum aegilopoides*. Another mutant, less drastic with regard to earliness, was more vigorous. The hereditary background of earliness in barley has been explored by a great many geneticists. Summarizing the data available up to that time, SMITH (1951, p. 167) stated that earliness may be dominant or recessive and depend on one, two, three or many genes. WEXELSEN (1934) found evidence of linkage of one factor for earliness with row number (linkage group I, SMITH, in Chromosome 2) and of linkage between another earliness factor and factors for rough awns and dense ears (linkage group V, SMITH, in Chromosome 7). Possibly another factor for earliness lies in linkage group IV (SMITH), which is carried by Chromosome 4. Apparently a series of gradations exists with regard to earliness. GUSTAFSSON (1947, p. 62) described a large-kernelled mutant of Golden barley with increased yield and enhanced earliness (one day). The drastic mutant erectoides 16 mentioned above was found to decrease yield by some 5—10 per cent. In the Gatersleben assortment of induced mutants (SCHOLZ and LEHMANN, 1958, 1959) there are also mutants that increase earliness. In his paper of 1957 SCHOLZ specially studied the production capacity of numerous mutants of this kind (p. 230, 232). Generally the very early mutants decrease yield considerably. The slightly changed mutants, on the other hand, largely keep the production capacity of the mother strain, or even improve it.

In his studies of the environmental influences on mutant viability in *Antirrhinum*, BRÜCHER (1943) mentioned two drastic mutants: *aestiva* and *matura*. These and other cases were discussed by SCHICK (1934) and STUBBE (1941). Mutations that modify earliness are certainly not at all rare, since earliness will often arise as a sort of by-product in connection with other changes in morphology and physiology.

Early mutants also occur in polyploid organisms like oats and wheat.

MACKEY (1956) reported a specially interesting case in oats. Four different mutants, arisen out of the same X-irradiated kernel, ripened four—five days earlier than the mother variety (Klock II). They are semi-drastic in behaviour. One of them, early 1, produced 3 per cent more grain than Klock II, being quite stable in other properties. The earliest mutant type, early 2, yielded 22 per cent less than Klock II and was markedly unstable, at least with regard to the white-kernel factor. In addition, MACKEY found an early mutant of Kolben spring wheat which produced 3 per cent more than the original variety.

Early mutants have been induced in sweet lupines (*Lupinus luteus*, TEDIN, 1954). In Sweden, where the species in question occurs at its northern limit of cultivation, such mutants may appear important and of direct agricultural value if yielding satisfactorily. The mutants concerned are under continuous testing.

The examples given refer primarily to some commonly cultivated plant species. Numerous others could be chosen. Suffice it to state that there seems to be a general possibility of inducing early mutants in diploid as well as polyploid species, both of the modifying and drastic type. Which kind will be most valuable from a practical point of view depends on the properties of the mother variety used, the changes required and the phenotypic expression of the induced alleles of the various gene loci. Even if they have no immediate value, many early mutants, both of the modifying and drastic type, may be utilized as parent types in the continued crossing program of a species.

Earliness is an important feature also under natural conditions. This has repeatedly been emphasized by TURESSON in his studies on ecotype formation (for instance TURESSON, 1930). The general belief that evolution largely proceeds by the successive accumulation of "small" mutations has been criticized by GUSTAFSSON (1954), who used the erectoid mutations as a model. The data, presented in this paper too, argue for the importance of both modifying and drastic mutations. Population composition does not change according to one principle only. Every possibility of variation will be utilized. If a "large" mutation has once arisen and if it is reasonably viable, in the heterozygous or the homozygous state or both, it can rapidly become the starting point for a new trend of variation by means of further mutation and recombination. In the case of a suitable environment, the new variation suddenly arisen may lead to an explosionlike widening of the range. Such has been the case in many agricultural plants. The alcaloid-poor mutants in *Lupinus* species, isolated by SENGBUSCH more than thirty years ago, suddenly

gained a wide area of cultivation. The same holds good for other plants where mutations in earliness provide new opportunities in agriculture and often eliminate the risks of cultivation in regions with a harsh climate.

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MULTIPLE ALLELISM IN INDUCED CHLOROPHYLL MUTANTS

II. ERROR IN THE AGGREGATION OF THE LAMELLAR DISCS IN THE CHLOROPLAST

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INTRODUCTION

IN a previous communication (v. WETTSTEIN, 1960 a) the induced chlorophyll mutants *xantha*-12, *xantha*-15 and *xantha*-18 in barley were shown to be allelic. The results of an electron microscopical investigation of plastids in these three mutants will be presented here.

Chlorophyll mutants, or chloroplast mutants, as they should rather be called, are blocked along different biochemical pathways. In favourable cases the step which cannot be carried out as a result of the gene mutation can be analysed, especially when it is concerned with the synthesis of chlorophylls and carotenoids (BOGORAD and GRANICK, 1953; GRANICK, 1954; CLAES, 1954, 1956, 1959; RÖBBELEN, 1956; SMITH, DURHAM and WURSTER, 1959; v. WETTSTEIN, 1959 a; ANDERSON and ROBERTSON, 1960; FALUDI, DANIEL and KELEMEN, 1960). A block in the synthetic pathways of the chloroplast constituents also arrests the differentiation of chloroplast structure such as the formation of the lamellae or grana. The effect of a mutant gene on the development of submicroscopic structure in plastids can be studied directly with the electron microscope and the block can be characterized (BOGORAD *et al.*, 1958; LEFORT, 1957, 1959; RÖBBELEN, 1959; SAGER, 1958; v. WETTSTEIN, 1954, 1957, 1957/59, 1958, 1959 b).

According to the "one gene — one function" hypothesis (BEADLE and TATUM) allelic mutants are concerned with the same step in a synthetic pathway. Recently this concept has evolved to the view that the structure of the functional unit of the chromosome (cistron) determines the structure of proteins *e.g.* the base sequence of desoxyribonucleic acid may determine the amino acid sequence in proteins.

Therefore we inquired whether alleles also cause the same structural block. Since we are presently in principle able to study the tertiary and supramolecular structure of proteins in the electron microscope a comparison of the effect of these three alleles on the differentiation of the lipoprotein layering in the developing chloroplast seemed of interest.

Pieces from 10—15 cm long primary and 5—10 mm long secondary leaves of the mutant seedlings from standard green-house tests (12—15° C) were fixed with 2 % buffered K-permanganate, dehydrated in increasing concentrations of acetone and embedded in araldite. Sectioning was carried out with an ultramicrotome of our own construction using thermal advance and magnetostriction. The micrographs were taken with a Siemens Elmiskop I.

THE STRUCTURAL BLOCK IN THE THREE ALLELIC MUTANTS

The three lethal mutants *xantha*-12 (Figs. 3—4), *xantha*-15 (Figs. 1—2), and *xantha*-18 (Figs. 5—6) show at least qualitatively the same aberrant differentiation of their chloroplast structure. The different steps in normal chloroplast development in barley have been described earlier (v. WETTSTEIN, 1957, 1957/59, 1958, 1959 b) and for details the reader is referred to these publications. (Cf. also MÜHLETHALER and FREY-WYSSLING, 1959 for discussion of structure and plastid development in other plants). The first step is the formation of vesicles from the inner of the two surrounding membranes of the proplastid. This vesicle formation can be carried out by the mutants (Figs. 3—5). The second step consists in the construction of the primary layers whereby the vesicles generally line up in a widely spaced concentric arrangement. Figs. 1, 2 and 5 reveal that the mutant plastids are capable of producing the primary layers. In darkness or low light intensity, as well as in many mutants grown under high light intensities the plastids progressively accumulate the vesicles in prolamellar bodies (v. WETTSTEIN, 1957, 1959 b). In *xa*-12, -15 and -18 a tendency toward this behaviour is also recognizable, especially in the younger plastid stages from the secondary leaves. Some vesicle accumulation remains in the older chloroplasts of the primary leaves (Figs. 3, 6). These prolamellar bodies are however always very loose, and they seem to disperse again just as in plastids from normal plants grown under low light intensity or in etiolated plastids exposed to light.

In a third step of the developmental sequence, discs are built up by the fusion of vesicles, and simultaneously, aggregation of these discs

in the primary layers forms the grana structure. The further development of the chloroplast is characterized by an increase in the number of discs and grana, an increase of the height of the grana packages and finally the establishment of a continuous lamellar system, whereby the grana are connected by unpaired double lamellae. For comparison the normal structure of the lamellar system in a chloroplast of a 10 cm long primary leaf is given in Fig. 7. As pointed out elsewhere (v. WETTSTEIN 1959 b, 1960 b; cf. also FREY WYSSLING and MÜHLETHALER, 1959) there is only one type of lamellae about 30 Å thick to be seen in micrographs. These are always membranes of discs and the grana structure is characterized by an intimate pairing of these discs, whereas the membranes connecting the grana (being themselves large discs) are unpaired. As can be seen also in Fig. 7 grana aggregation can take place at any region of the discs.

The three allelic mutants under discussion form along the primary layers packages of two to ten regularily aggregated discs, which could be called grana (Figs. 1—6). The plastids in all the pictures show that the vesicles can fuse into larger units, whereby discs can be formed. It can also be seen from these micrographs and especially from Fig. 5 that the fused vesicles or discs aggregate into the grana-like lamellar packages in these mutant plastids.

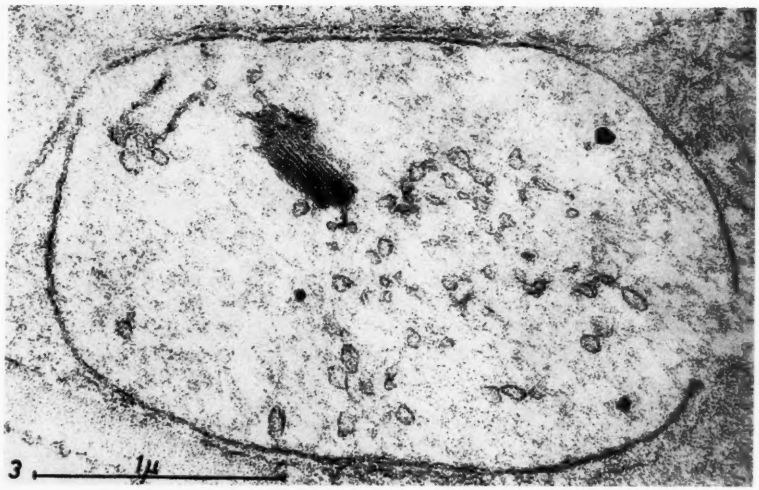
The mutant "grana" however differ in three ways from the grana in normal chloroplasts of corresponding age. 1) They are fewer in number. 2) They are much larger in diameter than grana in wild type barley chloroplasts. In extreme cases (Fig. 2) a mutant "granum" may extent nearly throughout the whole plastid. 3) The fine structure of the disc association is different from the normal grana arrangement.

ON THE NATURE OF THE ERROR IN THE LAMELLAR ORGANIZATION

Let us now examine the structure of the grana-like aggregates of lamellar discs as compared with the wild type more closely. The grana are considered columns of piled up discs. If grana are cut perpendicular to their longitudinal axes they would appear as circles containing homogeneous material in micrographs. The chances of obtaining sections precisely in this direction are small, but frequently more or less oblique sections through grana show part of a disc membrane as a homogeneous rounded area. That also the grana-like structure of the mutants are made up of a pile of discs can be seen from Fig. 4, which contains such an oblique cut. The homogeneous area of part of a

Fig. 1. Chloroplast in 10 cm long primary leaf of *xantha*-15. — × 57 000.

Fig. 2. Lamellar package and vesicles in chloroplast of *xantha*-15. — × 55 000.



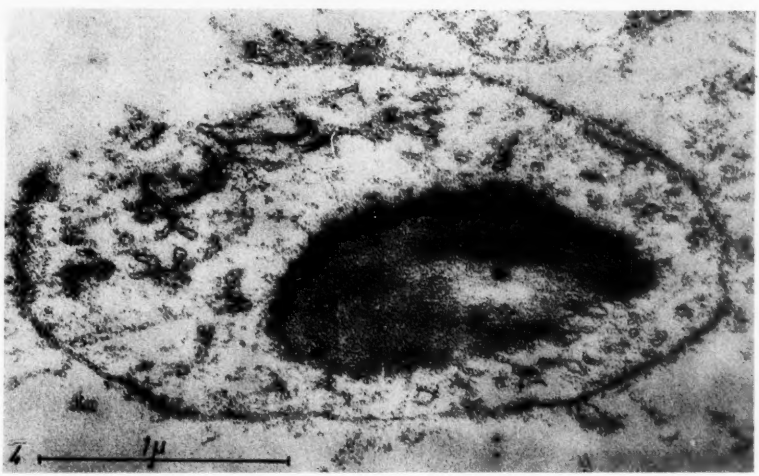
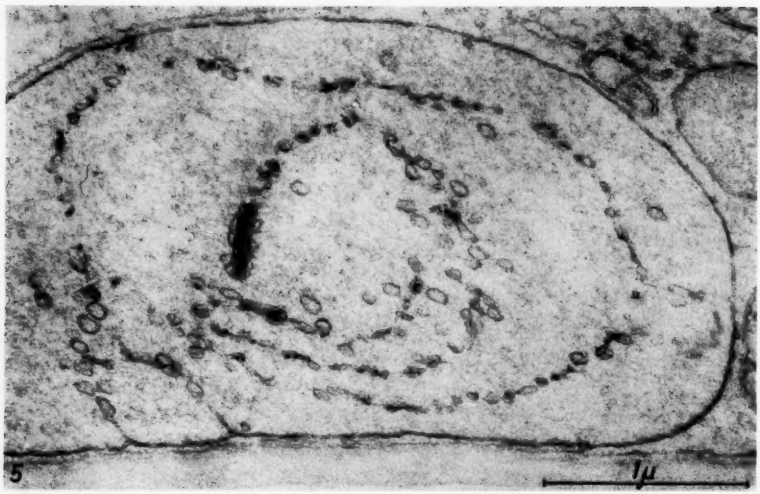


Fig. 3. Section through chloroplast in primary leaf of *xantha*-12. — × 39 000.
Fig. 4. Chloroplast of *xantha*-12 with obliquely sectioned lamellar package. — × 39 000.



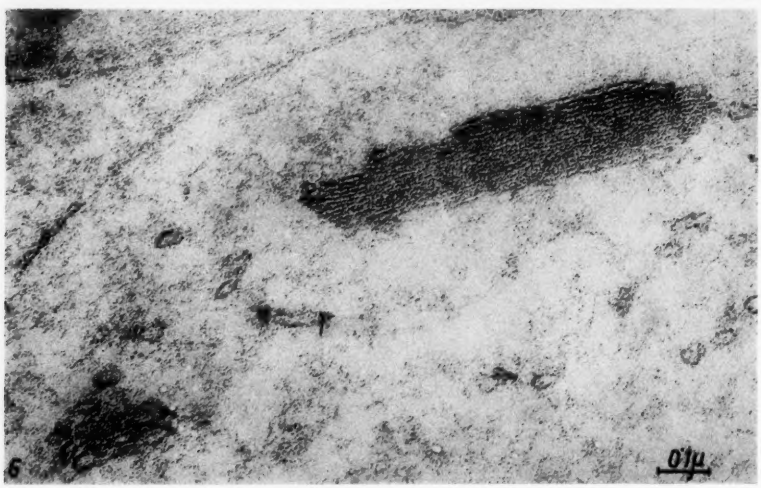


Fig. 5. Chloroplast of *xantha*-18 with vesicle formation by invagination of the inner surrounding membrane and small lamellar packages. — × 31 000.
Fig. 6. Lamellar package and aggregated vesicles in plastid of *xantha*-18. — × 80 000.

Fig. 7. Lamellar structure of normal chloroplast in barley. — × 125 000.

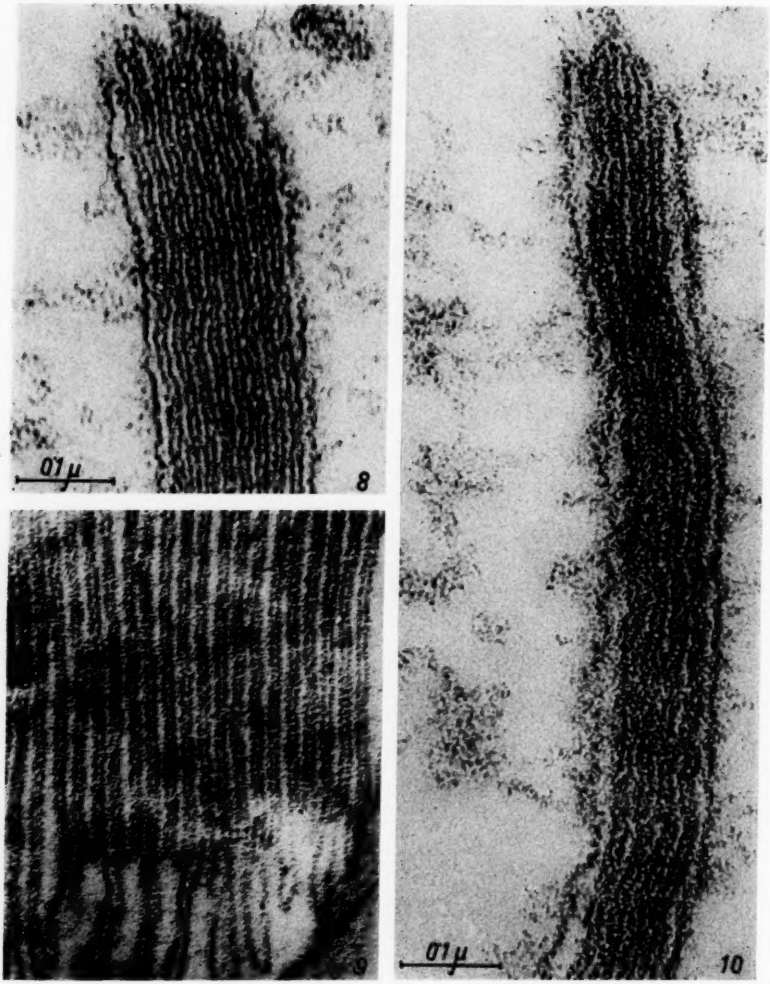


Fig. 8. Section through lamellar package of *xantha*-15. — × 150 000.
Fig. 9. Section through granum of normal chloroplast. — × 150 000.
Fig. 10. Section of lamellar package of *xantha*-15. — × 155 000.

strongly electron scattering membrane is apparent. In the same plastid a net-like structure lies to the left of the lamellar aggregate. This can be interpreted as a section of the primary layer in its plane showing the fusion of vesicles and the formation of discs.

Higher magnifications of the normal grana structure are presented in Figs. 7 and 9 and can be compared with the aggregates of discs in the mutant plastids of Figs. 8 and 10. It can be seen that the thickness of the lamellae and the number of lamellae or discs per unit length are the same in normal grana and in the mutant aggregates. An important feature of the grana structure is that the layer between the intimately aggregated discs is more strongly electron scattering than the space within the discs. Also the mutant aggregates show more electron scattering between than within the discs.

The mutant aggregates, however, have a less close association of their discs than the grana. The distance between the discs in a normal granum is smaller than the thickness of the space inside the disc, which gives rise to the picture of a pronounced pairing of lamellae in the grana (Figs. 7 and 9). In the mutant aggregates all lamellae appear to be equally spaced, the layer between the discs being as thick as the space inside the disc. (The space inside the two outermost discs is frequently larger than that of the others, both in normal grana and mutant aggregates. This is probably due to swelling during the fixing and embedding procedure.)

The dimensions as they were calculated from measurements on micrographs are given in Table 1. The thickness of the lamellae in wild type plastids has been measured to about 40 Å, those of the mutant aggregates to about 30 Å. These values agree within the errors inherent in the determination, the values being dependent on the reproducibility of the magnification in the microscope (around 10 % error), on the focus and on the precisely longitudinal direction of the cut through the

TABLE 1. *Dimensions in normal and mutant plastids.*

Dimensions	wild type	*xantha* -12, -15, -18
Thickness of lamellae	40±10 Å [1]	30±10 Å
Thickness of space within discs	85±10 Å	65±10 Å
Thickness of space between discs	20±10 Å	65±10 Å

[1] =range of variation.

lamellar structure. The thickness of the space within discs is approximately 85 and 65 Å for normal and mutant discs respectively. It is not possible from the present material to decide whether the mutant aggregates really contain thinner discs as would be expected if the number of discs per unit height is the same as in normal grana. The discs in the stroma as well as in the grana are very sensitive to swelling caused by the fixation and embedding, and differences as large or larger than the ones found here have been obtained in different preparations of normal chloroplasts. Further studies on this point are necessary. The difference in the thickness of the layer between the discs in normal and mutant plastids — about 20 and 65 Å respectively — is considered significant. There is very little variation in this respect in normal chloroplasts and the distance between the discs as well as the aggregation as such, is insensitive to different treatments of the chloroplast.

DISCUSSION

Apart from the fact that the lamellar aggregates in the mutants are of larger diameter than in normal plants the results can be schematically summarized as in Fig. 11. The error in the mutants as compared to the wild type is expressed in a more remote binding of the lamellar discs. From the observations it may be concluded that these mutants produce abnormal "grana" and that their plastids either lack a compound or message necessary to insure the normal grana structure, or manufacture an aberrant macromolecular constituent preventing the normal aggregation of the grana discs. The three mutant alleles of this gene concerned with grana construction produce the same type of abnormal aggregation of discs but this investigation is insufficiently advanced to answer the question whether quantitative differences in the effect of different alleles exist.

The aggregation of lamellar discs in grana formation is observed only in the presence of chlorophyll and correlated with chlorophyll synthesis (v. WETTSTEIN, 1958, 1959 a, b; v. WETTSTEIN and KAHN, 1960). In contrast to the general opinion that chlorophyll is necessary for the formation of lamellae (e.g. SAGER, 1958; RÖBBELEN, 1959) evidence exists that lamellar discs can be made in plastids grown in absolute darkness and in mutant *xantha*-10, where chlorophyll synthesis is blocked (v. WETTSTEIN, 1958). This may indicate that chlorophyll is contained in the thin layer which binds the lamellar discs together. *Xantha*-12, -15

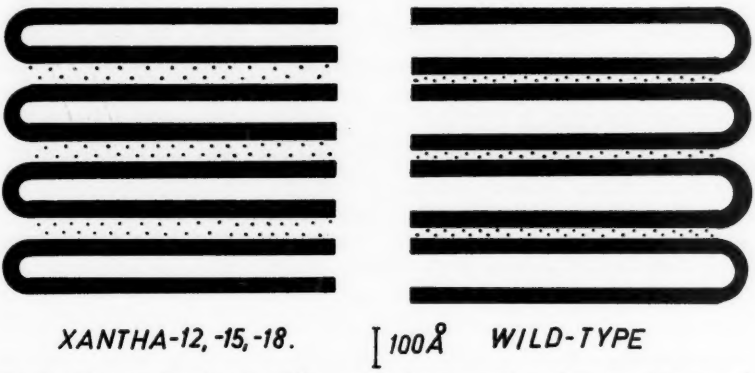


Fig. 11. Diagram showing the difference in the aggregation of the lamellar discs in chloroplasts of mutants *xantha*-12, -15, -18 and wild type.

and -18 can synthesize a good deal of chlorophyll. The error in aggregation then may be due to the lack of another substance in the layer between the discs or to a surface structure of the lamellae, which does not permit the close binding as found in normal grana.

Further analysis of the biochemical situation in these mutants is necessary. Since in a few cases lethal chlorophyll mutants seem to be able to form a normal chloroplast structure by supply of single amino acids and thereby become viable (WALLES, unpublished), feeding studies with amino acids of the three allelic mutants were tried, but no response has been obtained so far (WALLES, unpublished).

The investigations of abnormal human haemoglobins caused by single gene differences have provided us with knowledge on the effect of mutant genes on the primary structure of proteins. Haemoglobin C, S, G, and E seem to differ from normal haemoglobin A by the exchange of a single amino acid in the N-terminal β-chain of the globin (HUNT and INGRAM, 1959 a; HILL and SCHWARTZ, 1959; HUNT and INGRAM, 1959 b). In addition the position of the exchange in the amino acid sequence could be determined. Haemoglobin C and S revealed both an exchange in the 6th, haemoglobin G in the 7th and haemoglobin E in the 26th amino acid of the β-chain (BRAUNITZER, HILSCHMANN and MÜLLER, 1960). It is perhaps of significance that the genes for C and S are considered allelic, whereas the allelic relationship of the genes for G and E are not clear yet, but evidence for close linkage exists. Recent studies on induced mutants of tobacco mosaic virus have

furnished an example of changes in the amino acid composition of the coat protein (TSUGITA and FRAENKEL-CONRAT, 1960). In another mutant no difference in the protein composition was found (WITTMAN, 1959).

In some cases such a change in the amino acid sequence may lead to a change in the folding of the polypeptide chain and the tertiary structure of proteins (PAULING, 1958). It may be speculated that such abnormalities in the chloroplast protein could give rise to an aberrant submicroscopic lamellar structure as found in the allelic *xantha*-mutants. If the interpretation of the facts presented is correct, the electron microscopic analysis of highly ordered structures in the cell will permit exploration of the effect of mutant genes and their alleles on the level of organization close to that so successfully studied by X-ray analysis (PERUTZ, ROSSMAN, CULLIS, MUIRHEAD, WILL and NORTH, 1960).

Acknowledgement: — This paper is dedicated to Professor GERT BONNIER on the occasion of his 70th birthday. His stimulating interest and generous support since my association with the Genetics Institute of the University has been a great asset during the development of this work, and I want to express sincere thanks.

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SUMMARY

The development of the submicroscopic chloroplast structure in the three allelic mutants, *xantha*-12, -15 and -18 of barley was studied. These mutants are blocked at the same stage of development and produce aberrant aggregates of lamellar discs. The latter are larger and their aggregation is more remote than in the grana of the wild type. This can be considered as an error in grana formation.

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STUDIES ON THE EFFECT OF HOMOZYGOUS AND HETEROZYGOUS *w*-ALLELES ON LONGEVITY AND MATING PREFERENCE IN DROSOPHILA MELANOGASTER

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IN a paper by BONNIER *et al.* (1959) a series of experiments were described, in which the effect of different *w*-alleles on the rate of development was studied. The scope of that investigation was to obtain some information concerning the influence on viability of different alleles of a single gene in homozygous and heterozygous condition. The rate of development was chosen as the criterion of viability differences, because of the experience from previous experiments (BONNIER and JONSSON, 1957; BONNIER *et al.*, 1958), where this factor was found to be highly sensitivity to genetic changes. As isogenic stocks containing different *w*-alleles were available, it seemed however to be of interest to extend this investigation to other tests measuring adaptive fitness. Two such tests were chosen for the present study, namely longevity and mating preference.

In order to avoid genetic changes in the stocks the present investigation was performed immediately following the ones on the rate of development.

I. MATERIALS AND METHODS

1. *Longevity*

Two experiments were made, one 1957 and one 1958. The stocks used were the same as the ones from the corresponding years in the investigation on the rate of development. For a detailed description of the synthesis of these stocks reference is made to the paper by BON-

NIER *et al.* (1959). The following points may be sufficient for the present purpose.

The 1957 stocks were isogenic and homozygous for the two large autosomes as well as the euchromatic part of the X, except for the distal end, extending proximally to a point between *w* (1.5) and *ec* (5.5). The different stocks contained the following *w*-alleles: w^+ (wild type), w^{co}, w^a and *w*. The isogenic part of the X came from the original *w*-stock.

In the 1958 stocks the same *w*-alleles were used, but the synthesis of these stocks differed in respect to the X chromosome. The non-isogenic part containing the *w*-alleles had been diminished and extended from a point between *w* and *ec* to a point between *rb* (0.8) and *w*. The maximal length of this part of the X was thus 4.7, while in the 1957 stocks the corresponding section was 5.5 units. The rest of the euchromatic part of the X was identical between the stocks and came from a wild type strain, "Algeria". The two large autosomes were also made isogenic between the stocks, but in this case they were heterozygous. The reason for using heterozugous autosomes is discussed by BONNIER *et al.* (1959).

All the experiments were performed in 25° C. Newly hatched flies (1—2 days old, the females being virgins) were put in vials, either 25 females + 25 males or only 25 females per vial. The vials were changed simultaneously three times a week. Each 7 days the flies were etherized and counted.

Four series of experiments were performed. In series 1, 2 and 3, stocks from 1957 were used and in series 4 the flies came from the stocks 1958.

Series 1. All combinations of heterozygous *w*-alleles were made. Of each genotype 10 vials were put with 25 females and 25 wild type males in each vial, the whole material thus being 250 females per genotype. In order to detect any maternal influences, the females used emanated from reciprocal parental crosses — five vials from each cross.

Series 2. Homozygous females were put with males of the same genotype. As before 25 females and 25 males were put together in each vial with 8 vials per genotype.

Series 3. Heterozygous females of all combinations were put without any males, 25 females in each vial. The same precautions considering the maternal influence as in series 1 was taken.

Series 4. This was a repetition of series 3 but with the use of the stocks from 1958.

2. *Mating preference*

The 1957 stocks were used for this study. As it is known that the discrimination in different matings is at least in the main performed by the females, the experiment only involved mating preference of the females presented with two types of males. Homozygous females were picked as virgins and kept for 2—3 days. They were then transferred to new vials with 5 females in each vial. To each vial 5+5 males of two genotypes were added. Four genotypes of males, wild type, w^{co}, w^{a}, and w, were tested in all combinations two and two with each of the homozygous genotypes of females w^{co}, w^{a}, and w. No wild type females were used as this would have involved a test of all offsprings. Two identical series were made and in each series three vials were put of each cross. They were changed after three days and the flies were discarded after another three days period. The relation between the two kinds of offspring was taken as an indication of the mating which had occurred.

II. RESULTS

1. *Longevity*

The mean duration of life in the different series is shown in Tables 1 and 2. As the reciprocal parental crosses did not reveal any maternal influence, the data from both crosses are pooled together in series 1 and 3. When comparing the major groups of series 1 and 2, that is A) heterozygous wild type females (1—3), B) heterozygous and phenotypically non wild type females (4—6), and C) homozygous females (7—9), the following results were obtained. The statistical treatment involved a comparison of the mean squares of "between major groups" and "between subsamples", the latter ones being the different genotypes within the heterozygous and homozygous groups of females. Although the wild type heterozygotes showed a somewhat longer life span than the non wild type heterozygotes, this difference is not significant. Nor are the values for the non wild type heterozygotes significantly higher than those for the homozygotes. The wild type heterozygotes differ however significantly from the homozygotes ($0.005 > P > > 0.001$).

It should be pointed out in this connection that, although the same number of females and males were used per vial, in the series with heterozygous females the males were of wild type, while in the series with homozygous females, the males were of the same phenotype as

TABLE 1. *Series 1 and 2. Mean duration of life in weeks of heterozygous and homozygous females, kept with males.*

25 females and 25 males were kept in each vial. The heterozygous females (1—6) were kept with wild type males and the homozygous females (7—9) with males of their own phenotype. The figures show the pooled data from 10 vials of the heterozygous genotypes and 8 vials from the homozygotes.

		Mean duration of life in weeks	
No	Female Genotype	♀♀	♂♂
1	$w+/w^{co}$	4.94	4.93
2	$w+/w^{a}$	4.81	4.39
3	$w+/w$	5.12	5.00
4	w^{co}/w^{a}	4.04	4.86
5	w^{co}/w	4.84	5.05
6	w^{a}/w	3.56	5.12
7	w^{co}/w^{co}	3.66	5.25
8	w^{a}/w^{a}	3.16	3.90
9	w/w	3.73	4.22

the corresponding females. It is however unlikely that the presence of non wild type males in the latter case would have contributed to the low values for the homozygous females.

Considering the results of the different alleles, it can be noticed that the mean duration of life of the combinations with w^{a} is unexpectedly low even in heterozygous conditions. There is also a difference for

TABLE 2. *Series 3 and 4. Mean duration of life of females, kept without males.*

25 females per vial. The figures show the pooled data from 10 vials of each genotype.

	Mean duration of life in weeks	
Female Genotype	Series 3 (1957)	Series 4 (1958)
$w+/w+$	—	6.57
$w+/w^{co}$	7.36	7.07
$w+/w^{a}$	5.91	6.55
$w+/w$	6.28	6.06
w^{co}/w^{a}	4.54	—
w^{co}/w	6.02	—
w^{a}/w	5.77	—

w^+/w^a between series 3 and 4. It should also be pointed out that the behavior of w^a was rather erratic in the experiments on the rate of development (cf. BONNIER *et al.*, 1959). For these reasons the safest conclusions will probably be obtained from w^{co} and w.

If the genotypes with w^a are excluded from series 1 and 2 there is still no difference between the wild type heterozygotes (w^+/w^{co} and w^+/w) versus the non wild type heterozygotes (w^{co}/w). w^{co}/w has however a significantly longer life span than either of the two homozygotes ($0.005>P>0.001$ for both comparisons).

It may be mentioned that the variance for the homozygous females was slightly higher than for the heterozygotes. The ratio of the mean squares within the genotypes for the homozygous versus the heterozygous wild type females and the homozygous versus the heterozygous non wild type females gives in both cases a slight significance (at the 5 per cent level). This is in agreement with previous results on the variability of homozygotes and heterozygotes (cf. RASMUSSON, 1952).

Turning now to the series 3 and 4 (Table 2), the mean duration of life was found to be considerably longer for unmated females than females put together with males. The longer life span of unmated *Drosophila* females has also been found by previous authors (KRUMBIEGEL, 1929; MAYNARD SMITH, 1958). This difference probably depends on a difference in metabolic activity, which for a long time has been known to influence longevity in many animals (for a general survey see *i.a.* ALLEE *et al.* 1949).

A comparison of heterozygous wild type females versus heterozygous non wild type females does not reveal any significant difference, although the values are somewhat larger for the former as in series 1.

If w^a is excluded from the comparison it is found that w^+/w^{co} and w^+/w has a longer duration of life than w^{co}/w in series 3. This difference is not significant between w^+/w and w^{co}/w, but is highly so when the latter one is compared to w^+/w^{co}. The mean duration of life of w^+/w^{co} and w^+/w is very similar in series 3 with stocks from 1957 as compared to series 4 with stocks from 1958. It is of interest that in both series w^+/w^{co} has a significantly longer duration of life than w^+/w. This would indicate that the dominance effect of the wild type allele is not complete.

Unfortunately an experiment with homozygous wild type females and wild type males of the 1957 stocks could not be used, as the results indicated some experimental error. The series 1958 were therefore

started in order to obtain some information concerning the longevity of homozygous wild type females. The wild type homozygous females did not differ significantly from the wild type heterozygotes, although the former was not superior as in the case with the rate of development (BONNIER *et al.*, 1958).

2. *Mating preference*

The number of offspring from the different matings is shown in Table 3. When interpreting these data, it should be mentioned that there is no simple relation between the mating and the type of offspring, as each mating gives rise to a batch of sperms, used for a number of eggs. For this reason there is a considerable variability within each cross. It is anyhow clear that the wild type males are definitely superior to all mutant phenotypes in this mating competition. Considering differences between the mutant alleles the results are not consistent enough for any safer conclusion.

SUMMARY AND CONCLUSIONS

Using stocks, which were isogenic for the two large autosomes as well as the X, except for a short section around the white locus, differences in the mean duration of life was found when different combinations of *w*-alleles had been introduced. Although there may be a relation between the amount of pigmentation and duration of life, the statistical treatment of the data did not give any conclusive evidence of this. The experiments by BONNIER *et al.* (1958) did indicate such a relationship, when the rate of development was studied. For reasons presented above, the genotypes with w^a may have been somewhat unreliable and therefore the results with w^+, w^{co} and w were considered safer. Considering the effect of the different combinations of these *w*-alleles the following results were found.

If the material is divided into three groups, the wild type heterozygotes, the non wild type heterozygote and the non wild type homozygotes the actual data on the mean duration of life is highest for the first group and lowest for the last group. In the series with females and males put together, the differences are however small and only significant between the wild type heterozygotes and the homozygotes. In the series with only females a comparison can be made between wild type and non wild type heterozygotes. Although the mean duration of life still has a higher value for w^+/w^{co} and w^+/w as compared to w^{co}/w, the difference is only significant for w^+/co versus w^{co}/w.

TABLE 3. *Mating preference. Number of offspring from matings with different males.*

The first line shows the six groups of competing males. In the left most column 1 and 2 indicate the two identical series of experiments. In each pair of numbers in the table the data from three vials are pooled together.

♀♀ \ ♂♂		w^+-w^{co}		w^+-w^a		w^+-w		$w^{co}-w^a$		$w^{co}-w$		w^a-w	
co/co	1	142	37	83	57	62	48	18	142	146	67	52	118
	2	184	52	147	109	94	65	—	—	—	—	71	29
a/a	1	124	40	157	89	142	61	66	80	0	168	59	111
	2	68	42	125	47	126	44	—	—	8	216	43	122
w/w	1	78	31	144	31	104	25	100	25	50	84	39	112
	2	81	9	69	79	46	29	28	9	45	102	56	60
	Total	677	211	725	412	574	272	212	256	249	637	320	552

Considering the possibility of a heterosis effect of the alleles, no superiority of wild type heterozygotes as compared to the homozygous wild type was found. On the other hand w^{co}/w lived significantly longer than either of the homozygotes w^{co}/w^{co} and w/w.

There is an indication that the dominance of the wild type allele is not complete, as w^+/co had a significantly longer duration of life than w^+/w in both the experiments with unmated females.

In the experiments on mating preference, the wild type males were preferred to any type of non wild type males by the females.

Acknowledgement. — The authors want to thank Professor BONNIER for many helpful discussions. At this occasion of his 70th anniversary it is also a pleasure for us to express our sincere gratitude for all the kind help and encouragement through the years and to express to him our best wishes for the future.

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EXPERIMENTAL STUDIES IN *HIERACIUM PILOSELLA* L.

I. REPRODUCTION, CHROMOSOME NUMBER AND DISTRIBUTION

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INTRODUCTION

THE Linnean *Hieracium pilosella* has given its name to several groups and subgroups within the genus. The genus is divided into two subgenera, *Pilosella* and *Archieracium*, and the *Pilosella* group again into a number of subgroups, one of them being the *Pilosellina* subgroup. The genus is further known to be excessively polymorphic, not least the Pilosellas, where apomixis of the apospory type with facultative sexuality prevails together with, as in some interesting forms of the group, obligate sexuality. The systematic treatment of the great number of "species" within the genus and in the Pilosellas is accordingly a most difficult problem confronting the taxonomist. After the appearance of the imposing monograph of the subgenus *Pilosella* by NÄGELI and PETER (1885) mostly Scandinavian botanists have been engaged in the *Pilosella* taxonomy, the outstanding names being DAHLSTEDT in Sweden, NORRLIN in Finland and OMANG in Norway. As a result of their diligent and painstaking work taxonomical units have been distinguished and named in this group by the hundred.

Our own studies have been limited to a rather narrow range of types, almost exclusively belonging to the collective species *H. pilosella* L., whose swarm of "species" makes up the subgroup *Pilosellina*. Most of these types have long and slender stolons, bearing small distant leaves, decreasing in size towards the stolon apex. The involucres are about 10 mm broad, their bracts up to 1.5 mm wide, acuminate. A smaller group of types differs from the preceding in having short and curved stolons, bearing homophyllous leaves with large apical rosettes. The

involucres are up to 14 mm broad and rounded below, their bracts up to 2.5 mm wide, rather obtuse. This latter group of types has been called *H. macrolepidium* NORRL. (=*H. peleterianum* AUCTT.). Accordingly the group *Pilosellina* is split by the taxonomists into the smaller group *Macrolepidia* and *Pilosella* in a stricter sense (and a third, viz. *Furcata*). The main bulk of our material belongs to the two first-mentioned *Pilosellina* groups.

A second Linnean species, *H. auricula*, belonging to the subgroup *Auriculina*, has been studied to a limited extent, while hybrids and intergradation between this species and *H. pilosella* L. occur in nature. The species has also been used in some of our crosses.

MATERIAL AND METHODS

The collecting work started 1944—45 and about 500 collections from nearly as many localities have been studied through the years. The localities are fairly well spread over the country; only very few collections come from abroad. In the collecting work we have received much help from friends and colleagues, and we wish to express our sincere thanks for this valuable assistance.

The transplants were all grown in the Botanical-Genetics garden of the Royal Agricultural College at Uppsala. They were allowed to grow in plant boxes in the open until new stolon rosettes had been developed. One rosette from each transplant was then potted and put in a cold-house for chromosome counts and crossings, while the remaining rosette material was left in the plant boxes for studies in the following seasons with regard to flower colour, stolon characters, hairiness, time of flowering etc. The chromosome counts, based on root tips, were made in the traditional way; the roots were fixed in Karpechenko, cut, and stained with crystal violet. The staining and the countings did not meet with any difficulties.

The open-ground cultures were also used in the castration experiments. Five to ten flower heads in each transplant number were cut with a razor in such a way that only the lower part of the head remained, while the upper part of the filaments and the styles together with the anthers and the stigmas were removed (cf. RAUNKIÆR, 1903; OSTENFELD, 1906). The proper time for the castration was found to be 1—2 days before the heads opened, the flower colour having just changed at that time from light green to yellow. In order to prevent any loss of achenes at the ripening the castrated heads were wrapped in thin

gauze bags. It should be added that castration does not seem to damage the flowers. In the apomictic types the castrated heads develop at least as many achenes per head as the unwounded ones. In studying this question, as well as the fertility of the sexual types, where castration has no effect, five to ten flower heads in each transplant number were harvested after free pollination in the open-ground cultures. As soon as these flowers were wiltering they also were wrapped in gauze bags to prevent loss of achenes.

EMBRYOLOGICAL NOTES

From the important work by ROSENBERG (1906, 1907) we know, that the apomictic mode of reproduction, occurring in subgenus *Pilosella*, rests on apospory. The species examined by ROSENBERG were *H. excellens*, *H. flagellare* and *H. aurantiacum*.

Embryological work in the subgenus was not resumed until 1933 when CHRISTOFF and POPOFF studied four different types of the subgenus. One of these, *H. Pavichii* HEUFF., closely related to *H. florentinum* N. et P., is a diploid amphimict. The other three, according to the authors typical *H. pilosella* L., do not even belong to the *Pilosellina* group, as is evident from their own description. The three types represent aposporous pentaploids, however.

GENTSCHEFF (1937) studied, among other species, also *H. pilosella*. He does not describe the plant, and it remains doubtful, whether it belongs to the subgroup *Pilosellina*. The embryo development is described for the first time in this subgroup, provided that the determination is correct. Also this type represents an aposporous pentaploid.

CHRISTOFF and CHRISTOFF (1948) studied *H. Hoppeanum* SCHULT., a *Pilosellina* species with 2n=45, closely related to *H. macrolepidium* but not occurring in Scandinavia. By colchicine treatment a C_0-plant with 2n=90 was produced, which in the offspring gave rise to 2n=90 plants as well as to 2n=45 plants. The type, as well as the doubled C_0-plant, were studied embryologically and were found to develop aposporous embryo sacs, while the original EMC degenerated at an early stage. In the aposporous *Hieracia*, we know, that certain normal EMC do not degenerate but give rise to chromosome reduced eggcells in need of fertilization. In this connection OSTENFELD (1910) should be mentioned, who obtained hybrids between *H. aurantiacum* and aposporous *H. pilosella* as mother. The work of ROSENBERG (1907), as is well known, showed the presence of normally developed embryo sacs in *H. excellens*.

CHRISTOFF and CHRISTOFF are of the opinion, however, that the meiosis found in the chromosome doubled C_0-plant takes place in somatic cells from the integument. "These somatic cells, which occupy the cavity of an apparently older ovule, probably appeared after the degeneration of the archesporium." They take for granted "that the embryos which gave rise to the individuals with the reduced chromosome number originate from somatic cells, which have passed through meiosis", while the 2n=90 plants come from ordinary, aposporous embryo sacs with the unreduced chromosome number.

However, since colchicine treated plants often give rise to chimeras, diploids and tetraploids may be produced from the same C_0-plant. In the red clover, for instance, it is not uncommon to find the one half of the head with diploid flowers, the other with tetraploid (unpubl.).

Presuming the chromosome doubled C_0-plant of *H. Hoppeanum* were a chimera no additional commentary is necessary to explain the occurrence of plants with 2n=45 in the offspring.

Since hardly any forms belonging to the subgroups here treated, viz. *Macrolepidia* and *Pilosella* s.str. have been examined in this respect, representatives of these subgroups have been checked embryologically. As was expected, apospory was also found to be typical of these groups. Forms or biotypes, which combine facultative sexuality with apomixis, as is the case in the aposporous Pilosellas, have been called *amphiapomicts* by the senior writer (TURESSON, 1926), and this term has, therefore, been used in the following.

TYPE, CHROMOSOME NUMBER AND DISTRIBUTION

Before discussing Tables 1—7 it should be observed, that the provinces with reported localities are arranged from South to North, and that the foreign collections are tabulated at the end of the table or chromosome group.

The basic chromosome number in subgenus *Pilosella* is 9. In the subgroup *Pilosella* the diploid, 2n=18, is represented by the sexual or amphimictic *H. macrolepidium* (see Table 1). Its main distribution in Sweden is in the southern and middle parts, especially in the coastal provinces, where it grows in open, grass-covered hills forming dense mats. The underside of the leaves is bright white through a dense and short coat of stellate hairs. The flower heads are rather large and bright yellow.

In *H. macrolepidium* also types with 2n=27, 2n=36 and 2n=45

TABLE 1. *Distribution of amphimictic H. macrolepidium.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
146	Landskrona 1	Scania	G. TURESSON	1949	18
148	„ 3	„	„	1949	18
180	Vitemölle	„	B. TURESSON	1949	18
252	Saxtorp 1	„	G. TURESSON	1950	18
253	„ 2	„	„	1950	18
254	„ 3	„	„	1950	18
331	Åhus 1	„	„	1951	18
332	„ 2	„	„	1951	18
333	„ 3	„	„	1951	18
334	Brösarp	„	„	1951	18
339	Lomma 1	„	„	1951	18
340	„ 2	„	„	1951	18
341	„ 3	„	„	1951	18
345	Kullen	„	„	1952	18
441	Beddinge 1	„	„	1952	18
442	„ 2	„	„	1952	18
443	Stavsten 1	„	„	1952	18
444	„ 2	„	„	1952	18
510	Kämpinge	„	„	1954	18
532	Stenshuvud 3	„	„	1956	18
356	Haverdal	Halland	„	1952	18
36	Gisebo	Småland	„	1945	18
86	Kalmar	„	N. ERLANDSSON	1949	18
456	Skatelöv	„	G. TURESSON	1953	18
84	Kastlösa	Öland	N. ERLANDSSON	1949	18
421	Vickleby	„	B. TURESSON	1952	18
424	Resmo 2	„	„	1952	18
426	„ 4	„	„	1952	18
428	Långe Jan	„	„	1952	18
429	Byerum	„	„	1952	18
430	Byxelkrok	„	„	1952	18
423	Resmo 1	„	„	1952	18
224	Gothem	Gotland	H. NORDENSKIÖLD	1950	18
225	Lickershamn	„	„	1950	18
227	Östergarn	„	„	1950	18
229	Hejnum	„	„	1950	18
230	Hoburg	„	„	1950	18
231	Stenkyrka	„	„	1950	18
537	Vestergarn 2	„	G. TURESSON	1957	18
543	Hoburg 1	„	„	1957	18
544	„ 2	„	„	1957	18
549	Högklint 2	„	„	1957	18
371	Trollhättan 1	Västergötland	„	1952	18

TABLE 1. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
372	Trollhättan 2	Västergötland	G. TURESSON	1952	18
373	„ 3	„	„	1952	18
473	Forshem	„	„	1953	18
90	Ringstad 2	Östergötland	„	1949	18
241	Strömstad 1	Bohuslän	„	1950	18
242	„ 2	„	„	1950	18
244	„ 4	„	„	1950	18
357	Kareby 1	„	„	1952	18
358	„ 2	„	„	1952	18
359	„ 3	„	„	1952	18
360	„ 4	„	„	1952	18
366	Bräckan, Orust	„	„	1952	18
53	Fiskebäckskil	„	„	1948	18
480	Sannahed	Närke	„	1953	18
94	Vik	Uppland	„	1949	18
187	L. Ullsjöfjärden	„	H. NORDENSKIÖLD	1950	18
192	Uppsala 3	„	B. TURESSON	1950	18
212	Vik 1	„	G. TURESSON	1950	18
214	Funbo 1	„	„	1950	18
260	Kvarnberget 2	„	„	1951	18
261	„ 3	„	„	1951	18
323	Skärplinge	„	„	1951	18
440	Ramsta	„	B. TURESSON	1952	18
438	Valsgärde 4	„	„	1952	18
508	Harg 1	„	„	1954	18
512	Fontainebleau	France	H. NORDENSKIÖLD	1954	18
268	Stenkyrka	Gotland	„	1950	27
475	Mariestad 2	Västergötland	G. TURESSON	1953	27
193	Uppsala 4	Uppland	B. TURESSON	1950	27
194	„ 6	„	„	1950	27
213	Vik 2	„	G. TURESSON	1950	27
410	Valsgärde	„	B. TURESSON	1952	27
176	Ölbäck	Gotland	B. PETTERSSON	1949	36
177	„	„	„	1949	36
223	„	„	H. NORDENSKIÖLD	1950	36

TABLE 2. *Distribution of amphiapomictic H. macrolepidium.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
141	Tydinge	Scania	B. LÖVKVIST	1949	36
468	Forserum	Småland	G. TURESSON	1953	36
42	Slite	Gotland	„	1947	36
228	Holmhällar	„	H. NORDENSKIÖLD	1950	36
540	Hoburg I	„	G. TURESSON	1957	36
541	„ II	„	„	1957	±36
50	Kristineberg	Bohuslän	„	1948	36
191	Uppsala 2	Uppland	B. TURESSON	1950	36
197	„ 7	„	„	1950	36
267	Väddö	„	H. NORDENSKIÖLD	1951	36
401	Biludden 1	„	G. TURESSON	1952	36
402	„ 2	„	„	1952	36
403	„ 3	„	„	1952	±36
404	Öregrund	„	„	1952	36
408	Rimbo	„	„	1952	36
435	Biludden	„	B. TURESSON	1952	36
505	Öregrund 1	„	„	1954	36
422	Vickleby 2	Öland	„	1952	45
470	Bjurum	Västergötland	G. TURESSON	1953	45
52	Lysekil 2	Bohuslän	„	1948	±45
245	Strömstad 5	„	„	1950	45
88	Virå	Södermanland	B. BERGMAN	1949	45
437	Valsgärde 3	Uppland	B. TURESSON	1952	45
439	„ 5	„	„	1952	45
168	Tällberg	Dalarna	„	1949	45

TABLE 3. *Distribution of amphimictic H. pilosella.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
1	Ljungbyhed	Scania	G. TURESSON	1944	36
6	Hylla	„	„	1945	36
8	Dalby	„	„	1945	±36
9	Simrishamn	„	„	1945	36
11	Gunnarp	„	„	1945	36
12	Sätaröd	„	„	1945	±36
14	Kungsmarken	„	„	1945	36
15	Ludvigsborg	„	„	1945	36
16	S. Rörum	„	„	1945	36
20	Östarp	„	„	1945	36
21	Stenestad	„	„	1945	36

TABLE 3. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
38	Tunbyholm	Scania	G. TURESSON	1945	±36
122	Kaffatorp 3	„	N. ERLANDSSON	1949	36
134	Broby 4	„	B. LÖVKVIST	1949	36
147	Landskrona 2	„	G. TURESSON	1949	36
150	„ 5	„	„	1949	36
152	Malen	„	„	1949	36
183	Hallands Väderö	„	B. LÖVKVIST	1949	±36
278	Axelvold 1	„	H. NORDENSKIÖLD	1951	±36
279	„ 2	„	„	1951	36
280	Knutstorp 1	„	„	1951	36
281	„ 2	„	„	1951	±36
293	Högestad 1	„	G. TURESSON	1951	36
294	„ 2	„	„	1951	36
295	Falsterbo 1	„	„	1951	36
296	„ 2	„	„	1951	±36
297	„ 3	„	„	1951	36
298	Skanör 1	„	„	1951	36
299	„ 2	„	„	1951	±36
311	Broby C	„	B. LÖVKVIST	1951	36
312	„ D	„	„	1951	36
315	„ G	„	„	1951	36
316	„ H	„	„	1951	36
317	„ I	„	„	1951	36
318	„ K	„	„	1951	36
329	Sånekulla	„	G. TURESSON	1951	36
330	Klagshamn	„	„	1951	36
335	Skillinge	„	„	1951	36
336	Sjöbo	„	„	1951	36
338	Bjerred	„	„	1951	36
343	Viktorshög	„	„	1952	36
346	Domsten 1	„	„	1952	36
347	„ 2	„	„	1952	36
445	Hohög	„	„	1952	36
446	Frostavallen	„	„	1953	36
447	Tjörnarp	„	„	1953	36
450	Vankiva 3	„	„	1953	36
451	„ 4	„	„	1953	36
483	Barsebäck	„	„	1953	36
487	Hovs Hallar	„	„	1954	36
488	Båstad 1	„	„	1954	36
489	„ 2	„	„	1954	36
529	Simrishamn	„	„	1956	36
533	Frostavallen	„	„	1956	36

TABLE 3. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
534	Haväng	Scania	G. TURESSON	1956	36
151	Högaskog 1	Halland	„	1949	36
354	„ 2	„	„	1952	36
33	Gränna	Småland	„	1945	36
467	Forserum 1	„	„	1953	36
498	Åby 2	Östergötland	„	1954	36
117	Talne 1	Denmark	S. O. BJÖRKMAN	1949	36
118	„ 2	„	„	1949	36
285	Rödding	„	H. NORDENSKIÖLD	1951	36
286	Viborg	„	„	1951	36
287	Ribe 1	„	„	1951	36
288	„ 2	„	„	1951	36
290	Skibsted	„	„	1951	±36
289	Lögstör	„	„	1951	36
291	Österild	„	„	1951	±36
292	Brömsholm	„	„	1951	36
257	Sussex 1	England	H. G. BAKER	1950	36
258	„ 2	„	„	1950	36
237	Obermoss, Zugsp.	Germany	H. NORDENSKIÖLD	1950	36

TABLE 4. *Distribution of amphiapomictic H. pilosella.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
3	Gyllebo	Scania	G. TURESSON	1945	45
10	Nybroån	„	B. TURESSON	1949	45
114	V. Ströö	„	B. LÖVKVIST	1949	45
127	Kaffatorp 8	„	N. ERLANDSSON	1949	45
132	Broby 2	„	B. LÖVKVIST	1949	45
133	„ 3	„	„	1949	45
135	„ 5	„	„	1949	45
136	„ 6	„	„	1949	45
137	„ 7	„	„	1949	45
138	„ 8	„	„	1949	45
139	„ 9	„	„	1949	45
153	Skälderviken	„	G. TURESSON	1949	45
155	V. Ströö 2	„	B. LÖVKVIST	1949	45
158	„ 5	„	„	1949	45
159	„ 6	„	„	1949	45
162	Kungsmarken	„	„	1949	45
184	Hallands Väderö 2	„	„	1949	45

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
309	Broby A	Scania	B. LÖVKVIST	1951	±45
310	„ B	„	„	1951	45
313	„ E	„	„	1951	45
314	„ F	„	„	1951	±45
319	„ L	„	„	1951	45
337	Bollerup	„	G. TURESSON	1951	45
344	Sibbarp	„	„	1952	45
452	Vittsjö	„	„	1953	±45
453	Osby	„	„	1953	45
282	Bräkne-Hoby 1	Blekinge	„	1951	45
283	„ 2	„	„	1951	±45
284	Karlskrona	„	„	1951	45
525	Tjurkö 1	„	„	1956	±45
527	„ 3	„	„	1956	±45
342	Tolarp	Halland	O. GELIN	1952	45
349	Skrea Strand 1	„	G. TURESSON	1952	45
350	„ „ 2	„	„	1952	45
351	„ „ 3	„	„	1952	45
352	Steninge 1	„	„	1952	45
353	„ 2	„	„	1952	45
355	Åsa	„	„	1952	45
490	Tönnersjö	„	„	1954	45
37	Visingsö	Småland	„	1945	45
78	Växjö	„	D. BERNTMAN	1948	45
218	Vassmolösa 1	„	B. TURESSON	1950	±45
219	„ 2	„	„	1950	±45
274	Jönköping	„	H. NORDENSKIÖLD	1951	45
275	Tånnö 1	„	„	1951	45
276	„ 2	„	„	1951	±45
277	Markaryd	„	„	1951	45
348	Lidnäs	„	G. TURESSON	1952	45
411	Hornsborg	„	O. GELIN	1952	45
432	Pataholm	„	B. TURESSON	1952	±45
454	Älmhult	„	G. TURESSON	1953	45
455	Råshult	„	„	1953	45
457	Urshult	„	„	1953	45
458	Rävamåla	„	„	1953	45
460	Lenhovda	„	„	1953	±45
461	Ryningsnäs	„	„	1953	45
462	Oskarshamn 1	„	„	1953	45
463	„ 2	„	„	1953	45
465	Vimmerby 2	„	„	1953	45
466	Eksjö	„	„	1953	±45

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
469	Bankeryd	Småland	G. TURESSON	1953	±45
491	Vrå	„	„	1954	45
492	Ljungby	„	„	1954	45
494	Bellö	„	„	1954	45
495	Svinhult	„	„	1954	45
515	Västervik, Gränsö 1	„	„	1956	±45
516	„ „ 2	„	„	1956	45
517	„ „ 3	„	„	1956	45
518	„ , Segersg. 1	„	„	1956	±45
519	„ , „ 2	„	„	1956	±45
520	„ , Kuggv.	„	„	1956	45
521	Oskarshamn, Gunnarsö	„	„	1956	45
522	„ , Rotvik 1	„	„	1956	±45
523	„ , „ 2	„	„	1956	45
524	„ , Saltö	„	„	1956	±45
119	Vickleby	Öland	N. ERLANDSSON	1949	45
425	Resmo 3	„	B. TURESSON	1952	45
431	Böda	„	„	1952	45
511	Djupvik, Föra	„	N. ERLANDSSON	1954	±45
41	Råby träsk	Gotland	G. TURESSON	1947	45
181	Fårösund	„	O. HEDBERG	1949	45
226	Lummelunda	„	H. NORDENSKIÖLD	1950	45
535	Lojsta Hed	„	G. TURESSON	1957	45
542	Hoburg 3	„	„	1957	45
545	Vike 1	„	„	1957	±45
546	„ 2	„	„	1957	45
547	„ 3	„	„	1957	±45
89	Ringstad 1	Östergötland	„	1949	45
92	Grensholmen	„	„	1949	45
112	Omberg 1	„	S. O. BJÖRKMAN	1949	45
113	„ 2	„	„	1949	45
178	Grensholmen	„	Å. GUSTAFSSON	1949	45
217	Borghamn	„	„	1949	45
496	Åtvidaberg	„	G. TURESSON	1954	45
498	Åby 1	„	„	1954	45
499	Näkna 1	„	„	1954	45
500	„ 2	„	„	1954	45
4	Göteborg	Västergötland	C. BLOM	1944	45
115	Läckö	„	S. O. BJÖRKMAN	1949	45
202	Hindås	„	H. NORDENSKIÖLD	1950	±45
203	Alingsås	„	„	1950	45
265	Tivägsboda	„	B. LÖVKVIST	1951	45
300	Falköping	„	G. TURESSON	1951	45

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
301	Mösseberg 1	Västergötland	G. TURESSON	1951	45
302	„ 2	„	„	1951	45
303	Kleva 1	„	„	1951	±45
305	„ 3	„	„	1951	±45
374	Trollhättan 4	„	„	1952	45
375	„ 5	„	„	1952	45
471	Bjurum 2	„	„	1953	45
474	Mariestad 1	„	„	1953	45
476	Undenäs	„	„	1953	45
477	Granvik	„	„	1953	45
51	Lysekil 1	Bohuslän	„	1948	45
56	Munkedal	„	„	1948	45
362	Stenungsund	„	„	1952	45
363	Svanesund	„	„	1952	45
364	Hårleby, Orust 1	„	„	1952	45
365	„ „ 2	„	„	1952	45
367	Svälte 1	„	„	1952	45
368	„ 2	„	„	1952	45
369	Uddevalla 1	„	„	1952	45
370	„ 2	„	„	1952	45
269	Laxarby	Dalsland	S. ODÉN	1951	±45
270	Ed, Stora Le	„	„	1951	±45
376	Mellerud 1	„	G. TURESSON	1952	45
377	„ 2	„	„	1952	45
381	Bengtsfors 1	„	„	1952	45
382	„ 2	„	„	1952	45
383	„ 3	„	„	1952	45
266	Säbylund	Närke	B. LÖVKVIST	1951	±45
271	Degerfors	„	S. ODÉN	1951	±45
478	Olshammar	„	G. TURESSON	1953	45
479	Askersund	„	„	1953	45
58	Kila 1	Södermanland	H. NORDENSKIÖLD	1948	45
59	„ 2	„	„	1948	45
91	Tystberga	„	G. TURESSON	1949	45
182	Sjösa	„	H. NORDENSKIÖLD	1949	45
259	Vaxåker 2	„	„	1950	±45
262	Södertälje	„	G. TURESSON	1951	45
273	Vaxåker	„	H. NORDENSKIÖLD	1951	45
307	Römora, Trosa archip.	„	„	1951	45
433	Sparreholm 1	„	B. TURESSON	1952	45
434	„ 2	„	„	1952	45
501	Simonstorp 1	„	G. TURESSON	1954	45
502	„ 2	„	„	1954	45

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
503	Klastorp	Södermanland	G. TURESSON	1954	45
504	Slemna	„	„	1954	45
79	Blankheden	Värmland	Å. GUSTAFSSON	1948	45
234	Noppen	„	„	1950	45
272	Bro	„	S. ODÉN	1951	45
386	Valnäs	„	G. TURESSON	1952	45
387	Karlstad	„	„	1952	45
388	Brattfors	„	„	1952	45
389	Saxå	„	„	1952	45
163	Harbo	Västmanland	B. LÖVKVIST	1949	45
390	Hjulsjö 1	„	G. TURESSON	1952	45
481	Arboga 1	„	„	1953	45
482	„ 2	„	„	1953	45
27	Bäcklösa	Uppland	„	1945	45
46	Lidingö	„	H. NORDENSKIÖLD	1948	45
48	Ärna	„	„	1948	45
95	Uddeboö	„	B. LÖVKVIST	1949	45
185	Hummeldal	„	G. TURESSON	1950	45
186	Torslunda	„	„	1950	45
189	Hjällstaviken	„	H. NORDENSKIÖLD	1950	45
190	Uppsala 1	„	B. TURESSON	1950	45
196	„ 7	„	„	1950	±45
205	Bogesund 1	„	Å. GUSTAFSSON	1950	±45
207	„ 2	„	„	1950	45
210	„ 3	„	„	1950	±45
215	Funbo 2	„	G. TURESSON	1950	±45
216	Gårdskär	„	B. LÖVKVIST	1950	45
220	Hammarby 1	„	G. TURESSON	1950	45
221	„ 2	„	„	1950	45
222	„ 3	„	„	1950	±45
255	Ösby	„	„	1950	45
256	Biludden	„	B. LÖVKVIST	1950	45
308	Ultuna	„	G. TURESSON	1951	±45
320	„	„	„	1951	±45
321	Vittinge 1	„	„	1951	45
322	„ 2	„	„	1951	45
324	Månkarbo 1	„	„	1951	45
325	Österbybruk	„	„	1951	45
326	Älvkarleby	„	„	1951	±45
406	Norrtälje	„	„	1952	45
407	Frötuna	„	„	1952	45
409	Gottröra	„	„	1952	45
413	Grisslehamn	„	B. TURESSON	1952	±45

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
416	Väddö Huvud 3	Uppland	B. TURESSON	1952	45
417	„ „ 4	„	„	1952	45
418	„ „ 5	„	„	1952	±45
419	Gottsunda	„	„	1952	45
396	Storvik 1	Gästrikland	G. TURESSON	1952	±45
400	Gävle	„	„	1952	45
61	Säter	Dalarna	„	1948	45
165	Hedemora	„	B. TURESSON	1949	45
392	Rämshyttan 1	„	G. TURESSON	1952	45
393	„ 2	„	„	1952	45
395	Hosjö	„	„	1952	45
70	Nordanede	Medelpad	Å. GUSTAFSSON	1948	45
72	Ånge	„	„	1948	45
75	Norrhassel	„	„	1948	45
174	Åre	Jämtland	G. TURESSON	1949	45
44	Vådö	Finland	H. NORDENSKIÖLD	1947	45
45	Östersundom	„	„	1947	45
63	Nåtö, Åland	„	E. HESSELMAN	1948	45
238	Fischbach, Styria	Austria	H. NORDENSKIÖLD	1950	45
239	Maria Trost, Graz	„	„	1950	±45
485	Katschberg	„	„	1953	±45
448	Vankiva 1	Scania	G. TURESSON	1953	54
528	Tjurkö 4	Blekinge	B. TURESSON	1956	54
464	Vimmerby 1	Småland	G. TURESSON	1953	54
420	Karlevi	Öland	B. TURESSON	1952	54
536	Vestergarn 1	Gotland	G. TURESSON	1957	±54
538	„ 3	„	„	1957	54
539	Eksta	„	„	1957	54
548	Högklint 1	„	„	1957	±54
60	Göteborg	Västergötland	„	1948	54
304	Kleva 2	„	„	1951	±54
378	Mellerud 3	Dalsland	„	1952	54
379	„ 4	„	„	1952	54
26	Bergshamra	Uppland	„	1945	54
77	Bogesund	„	„	1948	±54
405	Hallstavik	„	„	1952	54
414	Väddö Huvud 1	„	B. TURESSON	1952	54
415	„ „ 2	„	„	1952	54
399	Forsbacka	Gästrikland	G. TURESSON	1952	54
166	Hedemora 2	Dalarna	B. TURESSON	1949	54
97	Gånsvik 1	Ångermanland	G. TURESSON	1949	54
98	„ 2	„	„	1949	54
68	Östavall	Medelpad	Å. GUSTAFSSON	1947	54

TABLE 4. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
236	Lanser Alm, Innsbr.	Austria	H. NORDENSKIÖLD	1950	54
240	Glashütten, Koralpe	„	„	1950	±54
249	Stockacher Alm, Innsbr.	„	„	1950	±54
250	Polster, Hochschwab.	„	„	1950	±54
531	Stenshuvud	Scania	B. TURESSON	1956	63
131	Broby 1	„	B. LÖVKVIST	1949	63
526	Tjurkö 2	Blekinge	B. TURESSON	1956	±63

TABLE 5. *Distribution of amphimictic H. auricula.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
13	Järarna	Scania	G. TURESSON	1945	18
279	Axelvold 3	„	H. NORDENSKIÖLD	1951	18
154	V. Ströö 1	„	G. TURESSON	1949	18
156	„ 3	„	„	1949	18
120	Kaffatorp 1	„	N. ERLANDSSON	1949	18
83	Kastlösa	Öland	„	1949	18
49	Mörbylånga	„	H. NORDENSKIÖLD	1948	18
143	Gammelgarn 1	Gotland	B. PETTERSSON	1949	18
145	„ 3	„	„	1949	18
201	Hindås	Västergötland	H. NORDENSKIÖLD	1950	18
306	Kleva	„	G. TURESSON	1951	18
54	Munkedal 1	Bohuslän	„	1948	18
55	„ 2	„	„	1948	18
380	Bäcke	Dalsland	„	1952	18
57	Kila	Södermanland	H. NORDENSKIÖLD	1948	18
62	Vaxåker	„	„	1948	18
87	Virå 1	„	B. BERGMAN	1949	18
179	„ 2	„	„	1949	18
81	Medskogen	Värmland	Å. GUSTAFSSON	1948	18
211	Bogesund	Uppland	„	1950	18
200	Uppsala	„	B. TURESSON	1950	18
31	Bäcklösa	„	G. TURESSON	1945	18
25	Järlåsa	„	„	1945	18
397	Storvik 2	Gästrikland	„	1952	18
40	Tisjön	Dalarna	„	1945	18
167	Hedemora	„	B. TURESSON	1949	18
164	Älvdalen	„	„	1949	18
76	Ljusdal	Hälsingland	Å. GUSTAFSSON	1948	18
71	Ånge	Medelpad	„	1948	18

TABLE 5. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
69	Nordanede	Medelpad	Å. GUSTAFSSON	1948	18
71	Krångede	Jämtland	„	1948	18
73	Bräcke	„	„	1948	18
102	Ragunda	„	G. TURESSON	1949	18
105	Stugun	„	„	1949	18
64	Sundmo	Ångermanland	Å. GUSTAFSSON	1948	18
99	Gånsvik	„	G. TURESSON	1949	18
100	Sollefteå	„	„	1949	18
67	Östavall	Medelpad	Å. GUSTAFSSON	1947	18
80	Skellefteå	Västerbotten	„	1948	18
235	Lanser Alm, Innsbr.	Austria	H. NORDENSKIÖLD	1950	18
251	Moschkogl, Koralpe	„	„	1950	18
328	Patscherkofel, Innsbr.	„	„	1950	18

TABLE 6. *Amphimictic H. pilosella with some distinct H. macrolepidium characters.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
19	Brösarp	Scania	G. TURESSON	1945	27
121	Kaffatorp 2	„	N. ERLANDSSON	1949	27
123	„ 4	„	„	1949	27
149	Landskrona 4	„	G. TURESSON	1949	27
530	Stenshuvud 1	„	B. TURESSON	1956	27

TABLE 7. *Amphiapomictic H. pilosella with some distinct H. auricula characters.*

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
459	Kosta	Småland	G. TURESSON	1953	36
42	Slite	Gotland	„	1947	36
497	Norsholm	Östergötland	„	1954	36
493	Vrigstad	„	„	1954	36
263	Berga Färed 1	Västergötland	B. LÖVKVIST	1951	36
264	„ „ 2	„	„	1951	±36
385	Åmål	Dalsland	G. TURESSON	1952	36
172	Virå	Södermanland	B. BERGMAN	1949	36

TABLE 7. Cont.

Field No.	Locality	Province or country	Collector	Year of coll.	2 n
24	Dalarö	Södermanland	G. TURESSON	1945	±36
391	Hjulsjö 2	Västmanland	„	1952	36
30	Notholmen	Sthlm archip.	„	1945	36
384	L:a Ulleviksfjärden	Uppland	B. TURESSON	1952	36
505	Öregrund	„	„	1954	36
32	Sifferbo, Gagnef	Dalarna	G. TURESSON	1945	36
248	Stockacher Bach, Innsbr.	Austria	H. NORDENSKIÖLD	1950	±36
249	„ Alm, „	„	„	1950	36

occur. The triploid is amphimictic, although much sterile; only a few achens are formed in each head, and the plant is "thinner" and weaker than the diploid and has difficulty in holding its own in competition. The type, therefore, is rare in nature, although it is readily made artificially. The tetraploids and the pentaploids (see Table 2) are larger and more robust than the diploid. They have also longer stolons, although characterized by the distinctive marks of *H. macrolepidium* for the rest, viz. homophyllous leaves on the stolons with large, apical rosettes. The tomentum on the underside of the leaves is looser and coarser than in the diploid. They are rather rare in nature and grow in scattered individuals without forming close mats, preferably in crevices and on open stony ground. The tetraploids and the pentaploids are amphiapomictic, with the only exception of the tetraploid collection from Ölbäck on the island of Gotland, which represents a deviating, strictly amphimictic type.

The morphological differences between *H. macrolepidium* and *H. pilosella* have been pointed out in the introduction. The best distinguishing marks are the stolon differences and the flower colour, which is darker yellow in *H. pilosella*. The long, slender stolons in this species become still longer with increasing chromosome number, the scapes also increase in height, and the hairiness on the underside of the leaves becomes coarser and looser.

The few triploid types found among the collections (see Table 6) have several typical characters of *H. macrolepidium* and represent, no doubt, hybrids between these species. They are, as in the case of triploid *H. macrolepidium*, fragile and weak and rather sterile. They are easily produced artificially.

We have not found any typical *H. pilosella* with lower chromosome

number than 2n=36, and this tetraploid is composed of strictly amphimictic types. All types with higher chromosome number (2n=45, 2n=54, and 2n=63, see Table 4) are found to be amphiapomicts. The only amphiapomictic tetraploids found show, without exception, typical characters of *H. auricula* (see Table 7), and represent hybrids between these species.

As seen from Table 3 the tetraploids are almost restricted in their distribution to the southernmost province, Scania. A few localities have been found north of this province (from southernmost Halland, from Småland and Östergötland) but it is of interest to find that these collections come from road-sides and not from undisturbed habitats. In Scania, however, these tetraploids are common, and they are not, as in the case of amphimictic *H. macrolepidium*, restricted only to the neighbourhood of the coast, but occur in the interior as well. The foreign material is very limited; two collections have been received from England and nine from Denmark (eight of these collections represent spread localities in Jutland, and the ninth, Brömsholm, comes from the east-coast of Zealand). It seems rather significant that these collections all represent the amphimictic, tetraploid *H. pilosella.*

Evidently, this type is rather common in Denmark, perhaps the predominating one. It is, then, surprising to find that the Danish material, used by OSTENFELD (1906) in his castration and crossing experiments and sent by him to ROSENBERG for cytological investigations, consisted of *amphiapomictic* tetraploids. OSTENFELD showed by castration its apomictic nature, and ROSENBERG (1917), in studying the pollen formation, found the chromosome number to be 2n=36. In the Copenhagen Botanical Museum dried and carefully numbered specimens are preserved of just the type used by OSTENFELD and sent to ROSENBERG. This type, however, is not a *H. pilosella* but a tetraploid *H. macrolepidium*, which is at once seen upon inspection.

The investigations of OSTENFELD and ROSENBERG, based on this comparatively rare type, have led to the erroneous conception that the "species" belonging to the subgroup *Pilosellina* are all apomictic and characterized by the chromosome number 2n=36. The inconstancy of many of these "species", as well as of the intergradation found, due to the presence of sexual types in the *Pilosellina* group, troubled the *Pilosella* specialists, knowing nothing of these sexuals, but well acquainted with the constant "species" of the strictly apomictic *Archieracia.* The false analogy led to the great number of so-called species, distinguished and named in the Pilosellas.

The pentaploid *H. pilosella* is the most frequent type in the group *Pilosellina* in Sweden. It occurs from southernmost Scania to Jämtland to the North, although it becomes rarer towards its northern limit. Contrary to the other types within the group it is also found in woody regions in the interior. The long stolons and the tall scapes, especially marked at fructification, make the pentaploids better fitted to live in somewhat taller vegetation than the other types. They form mats, as the tetraploids, but they are less dense because of the greater stolon length. The variation is also considerable, in leaf shape as well as in hairiness. Sometimes also furcated flowering stems with dark-haired involucres are found among the pentaploids, indicating a probable hybridization with *H. auricula.*

The 2n=54 and 2n=63 types are rare, and they are usually found as isolated individuals. They are robust in growth, but slow in development, flowering late and dispersing themselves with difficulty.

The amphimictic types of *H. macrolepidium* and *H. pilosella* are quite fertile with each other. Pure intermediates are seldom found; usually the forms are either *H. macrolepidium* with some *H. pilosella* characters, or *vice versa.* The rareness of the pure intermediates is probably due to the fact, that the hybrid is a rather sterile and weak triploid. With increasing chromosome number they represent amphiapomicts, showing a rather low frequency of hybridization in spite of being facultative sexuals and having good pollen.

The two sexual types can also be crossed with *H. auricula.* The amphimictic *H. auriculæforme* Fr. is a well known hybrid between *H. auricula* and *H. macrolepidium* with 2n=18 as in the parents. It is intermediate in habitus and not infrequently found in nature. A collection from Virå in Södermanland (coll. B. Bergman) has been studied.

In order to produce hybrids between types of subgroup *Pilosellina* and *H. auricula* some collections have been made of the latter species (see Table 5). The species prefer somewhat moister habitats than the members of the *Pilosellina* group, and has an extended distribution from South to North. Some collections from the Austrian Alps are also included in the table. All are amphimictic and found to have the chromosome number 2n=18 without exception.

SUMMARY

(1) The paper deals with representatives of the "species" of the two *Pilosellina*-subgroups of *Hieracium pilosella* L., viz. *Macrolepidia* and *Pilosella.*

(2) In amphimictic *H. macrolepidium* NORRL. the types 2n=18 (the most widespread), 2n=27 and 2n=36 (from only one locality) are found. In the amphiapomicts the types 2n=36 and 2n=45 have been found. The habitats and distribution of the different types are discussed.

(3) The amphimictic type in *H. pilosella* s.str. has the chromosome number 2n=36 and is largely distributed in southernmost Sweden. In the amphiapomicts the series 2n=45 (with the most extended distribution), 2n=54 and 2n=63 have been found. The habitats and distribution of the different types are discussed.

(4) The Danish *H. pilosella* type, used by OSTENFELD and sent by him to ROSENBERG for cytological study, preserved in the Copenhagen Botanical Museum, is not a *H. pilosella* s.str. but a tetraploid *M. macrolepidium.*

(5) Hybrids with *H. auricula* found in nature — amphimicts having 2n=18 and amphiapomicts with 2n=36 — have also been discussed.

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STUDIES ON CHROMOSOME 1 IN PISUM

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INTRODUCTION

FROM studies of translocations in *Pisum* involving chromosome segments carrying the genes *Gp* and *R*, respectively, LAMM (1951) suggested that these segments belonged to the same chromosome, *i.e.* chromosome 1. These suggestions were found to be correct in later investigations (LAMM, 1956; LAMM and MIRAVALLE, 1959).

In various translocations involving chromosome 1, increasing distance between these points of interchange and the gene *Gp* and decreasing distance to *R* was associated with an increasing tendency towards figure-of-eight formation whereas regular rings of four occurred in the reverse situation. Since the figure-of-eight formation depends on sufficiently long interstitial segments, *Gp* is more proximal to the centromere than *R*.

According to LAMPRECHT (1948) *Gp* is situated in chromosome V and *R* in chromosome VII. In spite of the results mentioned above he still claims that these genes in the normal structural type are situated in two different chromosomes (LAMPRECHT, 1958 and 1960). This opinion is partly based on mitotic studies made by BLIXT (1959). In the present paper, however, they have been disproved and new evidence has been obtained from meiotic investigations made on F_1 hybrids between LAMPRECHT's Lines 379 and 21. Seed of these lines has been supplied by the obliging courtesy of Mr. S. BLIXT.

THE CYTOLOGICAL TECHNIQUE

The cytological technique employed in the production of permanent slides for studies of meiosis in PMC's has been briefly described by LAMM and MIRAVALLE (1959). A more detailed description is given below.

Flower buds were first fixed in fresh 1 : 4 acetic-alcohol for four hours. If squashes were not to be prepared immediately, the buds were transferred to 95 % alcohol for about 12 hours and then stored in 70 % alcohol. The fixation of buds collected from field-grown plants in the hot days of July gave PMC's with darkly staining cytoplasm, which was inferior to that of buds collected during the winter from greenhouse-grown plants.

The technique used in preparing the squashes was as follows. One anther was removed from a bud under a preparation microscope. This anther was placed in a drop of iron-aceto-carmine of about 1 % carmine concentration on a slide. The PMC's were then teased out under the preparation microscope, every effort being made to remove all bits of anther wall and other debris that might hinder the spreading of the PMC's when pressure was applied later. Following this the PMC's were observed under low power to determine whether they were in the desired stage of division. If they were, a cover slip was placed on the drop and the distribution of PMC's viewed under the preparation microscope. Light topping on the cover slip with a curved needle was sufficient to separate the PMC's from one another. Blotting paper was then placed on the cover slip and steady downward pressure was applied with the fingers to spread and flatten the cells. Lastly, the PMC's were alternately viewed under high-dry power to determine the degree of spreading of the cells and staining of the chromosomes and cytoplasm and heated over a spirit lamp to aid both the spreading and staining. In order to make the slides permanent a modified McCLINTOCK schedule was followed. At first the cover slip was floated off the slide in 10 % acetic acid but later a quicker and more efficient method of removing the cover slips was employed. This method, which is described by CHRISTENSEN (1957, p. 28), consists first of freezing the cover slip to the slide after which it is removed by slipping a razor blade under one of its edges and gently peeling it off. The removal of the cover slip should be accompanied by popping and crackling sounds of the slide has been properly frozen. An apparatus developed by CHRISTENSEN which makes use of the cooling properties of CO_2 gas released from a pressurized cylinder was used. One may also use frozen CO_2 (dry ice) but this is less economical. Much fewer cells were lost by the freezing method than by the older acetic acid method of removing the cover slip. Following this, both cover slip and slide were taken through the following schedule:

1. 1 part glacial acetic acid, 9 parts absolute alcohol ..... 5 mins.
2. „ „ „ „ „ 3 „ „ „ ..... 5 „
3. „ „ xylol, 1 part absolute alcohol ..... 5 „

The cover slip and slide were then recombined in a thin xylol-canada balsam mixture. Blotting paper was placed on the cover slip and pressure was applied with the fingers to remove air bubbles and excess mounting medium.

CYTOLOGICAL INVESTIGATIONS

In a recent paper by BLIXT (1959), idiograms are given of, among others, LAMPRECHT's Lines 21 and 379. According to these authorities

Fig. 1. Meiosis in two PMC:s of the F_1 hybrid between LAMPRECHT's Lines 21 and 379. The upper nucleus shows one regular open ring of six chromosomes and four bivalents. The lower nucleus shows six chromosomes forming a figure-of-eight, due to chiasmata in the differential segment, and four bivalents. This F_1 hybrid probably represents the combination T(1—2)c×T(1—3)b.

the former is homozygous for a translocation between chromosomes I and VII (carrying the segments *A-I* and *R-Bt*, respectively), and the latter a translocation between chromosomes III and V (carrying the segments *St-B* and *Ust-Gp*). From comparisons of the somatic idiograms of these lines with the normal structural type represented by LAMPRECHT's Line

110, BLIXT (1959) claims to have given the cytological proof of these genetical assumptions. Obviously two rings of four and three bivalents would then be expected as the maximum association at meiosis of the F_1 hybrid between Lines 21 and 379.

According to the results obtained by LAMM and MIRAVALLE (1959), chromosomes V and VII (according to the nomenclature used by LAMPRECHT) are, however, different segments of one and the same chromosome. Line 21, according to the nomenclature used by LAMM and MIRAVALLE (1959), should be registered as T(1—2)c and line 379 probably as T(1—3)b (anyhow as T, 1—3).

It should be mentioned that T(1—2)a corresponds to HAMMARLUND's *K*-line, T(1—2)b to PELLEW's Early Giant Rogue and T(1—3)b to NILSSON's Extra Rapide (see LAMM and MIRAVALLE, 1959). Line 379 has been selected from the cross WEIBULL's Ambrosia×WEIBULL's Extra Rapid (cf. LAMPRECHT, 1953) and this fact as well as its linkage relations suggest that it is probably structurally identical with NILSSON's Extra Rapid and in any case chromosomes 1 and 3 are involved.

Last winter reciprocal crosses between Lines 21 and 379 were kindly made for me by Mr. R. HOCHBERGS at the Institute of Genetics in Lund. Meiosis of the F_1 hybrids thus obtained have been studied by me in the summer of 1960.

As expected a ring of six chromosomes and four bivalents was found to be the maximum association at meiosis (see Fig. 1). Hence only three chromosomes are involved, viz., chromosomes 1, 2 and 3. Quite a number of the associations of six appeared as figure-of-eight formations (see Fig. 1 below), indicating that the differential segment of chromosome 1 must be of considerable length.

The seed- and pollen fertility of the hybrid was also studied. In 50 pods with altogether 376 ovules 138 were normally developed and 238 aborted, i.e. 37 % fertile and 63 % sterile. Of 649 pollengrains 36 % were fertile and 64 % empty, i.e. sterile. This level of fertility is remarkably high but was, for instance, also found in the F_1 hybrid T(3—4)a× ×T(3—5)a (cf. LAMM and MIRAVALLE, 1959, p. 423, Table 2).

GENETICAL INVESTIGATIONS

According to LAMPRECHT (1954 a) the gene *Wsp* in the normal structural type is situated to the left of the *R-Bt* segment, the approximate distances being *Wsp* — 34 % — *R* — 39 % — *Bt*. No linkage has been detected between *Wsp* and *Gp*. If the diagram of chromosome 1 given

by LAMM and MIRAVALLE (1959, p. 432, Fig. 15) is correct, strong linkage should be expected between any point of interchange in the neighbourhood of *R* and *Wsp*, especially with regard to the suggested localization of the centromere.

In 1957 cross No. 135/57 was performed between Line 87 (Thibet 7, T(1—3)a, *R Wsp*) and LAMPRECHT's Line 360 (the normal structural type, *r wsp*). In F_2, which gave 85 % germination, the following segregation was obtained.

39 fert. *R*	— 35 fert. *r*	— 80 semist. *R*	— 8 semist. *r*
58 „ *Wsp*	— 16 „ *wsp*	— 62 „ *Wsp*	— 26 „ *wsp*
88 R „	— 31 *R* „	— 32 *r* „	— 11 *r* „

Chi-square analysis has given the values below, each based on 1 DF.

χ^2 segregation		χ^2 linkage	
fert : semist.	1.2099	T(1—3)a-*R*	30.6255***
R : *r*	0.2057	„ -*Wsp*	1.3909
Wsp : *wsp*	0.0741	*R* - „	0.0027

There is thus as expected a strong linkage between T(1—3)a and *R* with a crossing-over value of 8.6 ± 3.05 % not significantly deviating from the value 5.5 ± 0.75 % obtained earlier (LAMM, 1951). This result is incompatible with the mapping of *Wsp* given by LAMPRECHT (1954a).

However, in LAMPRECHT's Cross No. 1062 (LAMPRECHT, 1955) between Line 25 [structurally T(1—2)c] and the normal structural type, there is a significant linkage between the point of interchange and *Wsp* ($\chi^2 = 10.6433^{**}$) corresponding to 26.7 ± 3.53 % crossing-over (in LAMPRECHT's paper probably wrongly estimated at 33.1 ± 4.90 %). From the paper just cited it appears that the linkage between T(1—2)c and *R* amounts to 7.8 ± 0.99 % (although this figure is somewhat uncertain due to disturbed *R*-segregation and heterogeneity). There is also a tendency towards linkage with the *Ust-Gp* segment based on the non-significant crossing-over values 35.8 ± 6.94 % for T(1—2)c — *Ust* and 32.8 ± 5.72 % for T(1—2)c — *Gp*.

The great discrepancies between the linkage values of *Wsp* with the two points of interchange in the neighbourhood of *R* on chromosome 1 as discussed above are rather puzzling. Further investigations are therefore requested. In those studies the possibility of unexpected results due to certain kinds of quasi-linkage should also be taken into consideration (see LAMM and MIRAVALLE, 1959, p. 429).

DISCUSSION

A coordination of the results obtained from various studies on translocations in *Pisum* has been urgently requested. The present investigation is entirely in line with those aims. The meiotic behaviour of the F_1 hybrid here described speaks definitely in favour of the opinion that *Gp* and *R* are both situated in chromosome 1. Further, they show that the results of the somatic investigations on Lines 21 and 379, published by BLIXT (1959), are misleading. Somatic investigations of this kind should at least in *Pisum* always be complemented by meiotic studies.

LAMPRECHT (1954 b) states that chromosome II (carrying, among others, the gene *Oh*) is not involved in any translocation. The basic translocation tester set given by LAMM and MIRAVALLE (1959), however, involves all the seven chromosomes. In this connection it should be mentioned that Dr. E. NILSSON (unpublished) found evidence of linkage between T(6—7)a (NILSSON's N II) and *Oh* in a small backcross progeny. This indication is further supported by the results of an F_2 analysis of 284 plants made by the author. Here the crossing-over between T(6—7)a and *Oh* amounted to 21.4 ± 4.41 %. Unfortunately there was a significant deviation in the ratio fertile : semisterile, making the result somewhat obscure.

The occurrence of large, more or less gene-empty segments in *Pisum* is according to LAMM and MIRAVALLE (1959) one of the obstacles as regards the mapping of the chromosomes. LAMPRECHT (1958) has questioned the occurrence of such segments. However, the *Gp-R* segment seems to belong to this category provided that it does not contain the gene *Wsp*. Apart from this no marker genes have so far been localized to chromosome 4 involved in the translocations T(3—4)a and T(1—4)a (see LAMM and MIRAVALLE, 1959).

In the progenies of translocation heterozygotes tertiary trisomics often occur. The primary trisomics, on the other hand, seems often to be less viable in *Pisum*. It should be of interest to know whether this is also true with respect to chromosome 4.

It would be interesting to follow up the mapping work on the basic translocation tester set especially with respect to the chromosomes 4, 6 and 7. The present somewhat uncertain guess is that 7 is the *Oh-K* and 6 the *Pl-Wlo* chromosome, but this suggestion may be wrong. This tester set ought to be tested also with LAMPRECHT's Line 110 serving as the standard for the normal structural type. In addition, a number of additional problems of both scientific and practical value ought to be

more thoroughly studied. Unfortunately I lack the necessary facilities for those projects, and apart from small notes this paper will probably be the final report of my studies in *Pisum*.

SUMMARY

Meiotic studies of the F_1 hybrid between LAMPRECHT's Lines 379 and 21 have been performed. According to the author this cross is probably of the type T(1—3)b×T(1—2)c whereas BLIXT (1959), using Roman numerals to symbolize the chromosomes, found it to be of the type T(III—V)×T(I—VII). The maximum association at meiosis was one ring of six chromosomes and four bivalents. This provides fresh support for the opinion that chromosomes V and VII are merely different sectors of chromosome 1, and that this chromosome contains both the genes *Gp* and *R*.

BLIXT (1959) made his studies on somatic chromosomes. The meiotic checking made by the author shows that these somatic investigations in *Pisum* may give incorrect and faulty results. The coordination of linkage groups with chromosomes made by BLIXT (1959) seems thus to be doubtful.

Genetical investigations of the linkage relations between *Wsp* and two points of interchange on chromosome 1 have given incompatible results and only more thorough investigations could give an acceptable explanation.

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STRUCTURAL HETEROZYGOSITY IN *SECALE KUPRIJANOVII*

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IN 1959 A. MÜNTZING visited the Institute of Plant Industry in Leningrad, where he had the privilege of receiving several valuable seed samples. Among this material the allopolyploid product *Triticum durum*+*Secale Kuprijanovii* was represented as well as one of its parent species, *Secale Kuprijanovii* GROSSH. This species of *Secale* is a wild perennial with brittle rachis. The strain obtained originated from Transcaucasia.

Seeds of the allopolyploid and its *Secale* parent were sown in April 1959. The plants of *Secale* developed well, survived the following winter and reached full maturity in the summer of 1960. A total of 17 plants were raised from 50 seeds. Of the allopolyploid a small number of seeds were first sown in April 1959. This resulted in a few plants giving mature seeds at the end of the summer. Part of these seeds were sown in the field in the autumn of 1959, but almost all the seedlings died during the winter. Therefore, new seeds were sown in the field in April 1960, and although the resulting plants reached the flowering stage very late, plenty of material was available for study in the summer of 1960.

N. J. HRISHI made fixations of meiosis of the allopolyploid as well as of its parent species. The results of a detailed analysis of this material will be published later on. The present report is restricted to some data obtained in *Secale Kuprijanovii*.

For studies of meiosis permanent Feulgen squash preparations were made from spikes fixed in acetic alcohol. Determinations of the percentages of good pollen were based on preparations of single anthers. From each plant 300 pollen grains were examined, the pollen being stained in a medium containing 1 % acid fuchsin (8 cc), glycerine (40 cc), phenol (20 cc), lactic acid (20 cc) and distilled water (20 cc).

CYTOLOGICAL OBSERVATIONS

Meiosis was studied in a total of 13 plants, the stage observed mainly being first metaphase. In one of the plants (no. 7) the only stage available was diakinesis, which seemed to be normal with 7 bivalents. In another plant (no. 18) $6_{II}+2_{I}$ was the most frequent configuration, but p.m.c. with 7_{II} were also often met with. All the other plants, however, were found to be heterozygous for a segmental interchange, an association of four chromosomes+five bivalents being the most frequent configuration.

The detailed analysis of chromosome pairing in this material was carried out by N. J. HRISHI, the data obtained being given in Tables 1 and 2. Looking first at Table 1, we find that plant no. 8 is the most uncomplicated one, the configuration $1_{IV}+5_{II}$ being found in 89.4 per cent of the p.m.c. In another 1.2 per cent the association of four had broken up into a trivalent plus a univalent, and in the remaining 9.4 per cent of the cells the configuration was $1_{IV}+4_{II}+2_{I}$, owing to nonconjunction in a chromosome pair not involved in the interchange. According to these values an association of four chromosomes was found in 100 per cent of the p.m.c. observed. Similar values were found in nine other plants (nos. 1, 10, 13, 14, 15, 16, 22, 23 and 25), but in all of these individuals less than 100 per cent of the p.m.c. contained associations of four chromosomes. In some cells there were 7_{II} or the configurations $6_{II}+2_{I}$ and $5_{II}+4_{I}$, the percentages of such cells ranging from 1.6 to 17.1.

Plant no. 11 was also found to have an association of four chromosomes in the majority of the cells, but cells with only bivalents, or a combination of bivalents and univalents, were almost equally frequent, the percentages of the two categories, with and without associations of four, being 51.6 and 48.4, respectively. As it is known that the chiasma frequency in population plants of rye may vary considerably (A. MÜNTZING and AKDIK, 1948) it might be presumed that plant no. 11 had a lower chiasma frequency than the other plants discussed, which would explain the lower frequency of "quadrivalents". This assumption is somewhat supported by the fact that in this plant the frequency of p.m.c. with univalents was fairly high. Thus, altogether 12.1 per cent of the cells represented the configurations $6_{II}+2_{I}$ and $5_{II}+4_{I}$, the corresponding value in the other plants with an association of four chromosomes being lower.

Some other striking figures are met with in plant no. 13. In this plant

TABLE 1. *Percentages of different chromosome configurations at first metaphase in a strain of Secale Kuprijanovii.*

Configuration	Plant number												
	1	7	8	10	11	13	14	15	16	18	22	23	25
$1_{IV}+5_{II}$	72.9	Diakinesis: probably 7_{II}	89.4	83.9	50.6	58.0	85.2	84.4	84.5	—	76.2	77.4	82.6
$1_{IV}+4_{II}+2_{I}$	16.0		9.4	—	1.0	38.0	3.3	5.8	—	—	15.2	—	8.1
$1_{III}+5_{II}+1_{I}$	6.2		1.2	—	—	2.0	—	4.2	10.4	—	7.0	5.8	—
Total	95.1		100.0	83.9	51.6	98.0	88.5	94.4	94.9	0.0	98.4	83.2	90.7
7_{II}	4.9		—	6.5	36.3	1.0	8.2	—	5.1	41.0	—	12.5	5.2
$6_{II}+2_{I}$	—		—	6.5	9.9	1.0	3.3	2.8	—	55.7	1.6	4.3	4.1
$5_{II}+4_{I}$	—		—	3.1	2.2	—	—	2.8	—	3.3	—	—	—
Total	4.9		0.0	16.1	48.4	2.0	11.5	5.6	5.1	100.0	1.6	16.8	9.3
Number of p.m.c. examined	81		85	124	91	100	108	70	116	183	66	102	86

TABLE 2. *Percentages of zig-zag and non-zig-zag configurations at first metaphase. Pollen fertility and seed setting.*

Configurations and fertility	Plant number										
	1	8	10	11	13	14	15	16	22	23	25
Non-zig-zag	33.3	31.3	16.7	43.5	9.1	41.7	18.2	16.7	40.0	18.8	7.2
Zig-zag	66.7	68.7	83.3	56.5	90.9	58.3	81.8	83.3	60.0	81.2	92.8
Number of p.m.c.	60	80	48	46	55	72	55	90	50	69	69
Per cent good pollen	72.0	78.0	75.0	80.0	89.0	75.0	82.0	82.0	68.0	87 0	92.0
Per cent seed setting	23.6	13.8	22.9	—	19.2	29.9	20.0	18.1	41.4	32.8	19.8

98 % of the cells contained the association of four chromosomes, but only 58.0 per cent had $1_{IV}+5_{II}$, 38.0 per cent representing the configuration $1_{IV}+4_{II}+2_{I}$. Also in plants 1 and 22 the frequency of this configuration was fairly high. At present no plausible explanation of the high frequency of this configuration in some of the plants can be given. It should be mentioned, however, that in some of the plants the occurrence of associations of 6 chromosomes was indicated, though no quite convincing cases of this kind could be found. Thus, further work is necessary to decide, whether only four chromosomes are involved in the segmental interchange, or if conditions in this respect are more complex.

It remains to consider plant 18, in which a total of 183 p.m.c. from two spikes were examined. No association of four was found in this plant, but on the other hand quite regular pairing with 7_{II} was observed in only 41.0 per cent of the p.m.c. The largest class (55.7 per cent) showed $6_{II}+2_{I}$, and in 3.3 per cent of the cells there were $5_{II}+4_{I}$. Though there is no clear evidence of structural heterozygosity in this plant, it is not certain that the chromosomes in each bivalent are quite homologous. The mode of meiosis in plant 11 strengthens this suspicion, this plant, as indicated above, being intermediate between regular chromosome pairing with only bivalents and regular occurrence of an association of four chromosomes.

Though, consequently, the distinction between the two categories may not be perfectly clear, it is obvious that at least 11 of the 13 plants examined are definitely heterozygous for a segmental interchange. This certainly strongly indicates a *balanced structural heterozygosity*.

The plants cytologically studied were also examined as to *fertility*, the values obtained being included in Table 2. The percentages of apparently good pollen grains were found to range from 68.0 to 92.0 in the translocation heterozygotes, the average value being 80.0. Plant no. 18, in which there was no association of four chromosomes, was found to be quite male sterile, but the other plant, no. 7, probably belonging to the same category, had 85 per cent good pollen. The number of plants available is too small to allow any conclusions as to a possible average difference in fertility between the translocation heterozygotes and the plants with regular or almost regular chromosome pairing. It is striking, though, that pollen fertility in the translocation heterozygotes is on an average rather good. This is connected with the fact that in the associations of four, zig-zag-arrangement of the chromosomes is predominant. As is evident from Table 2 the percentages of

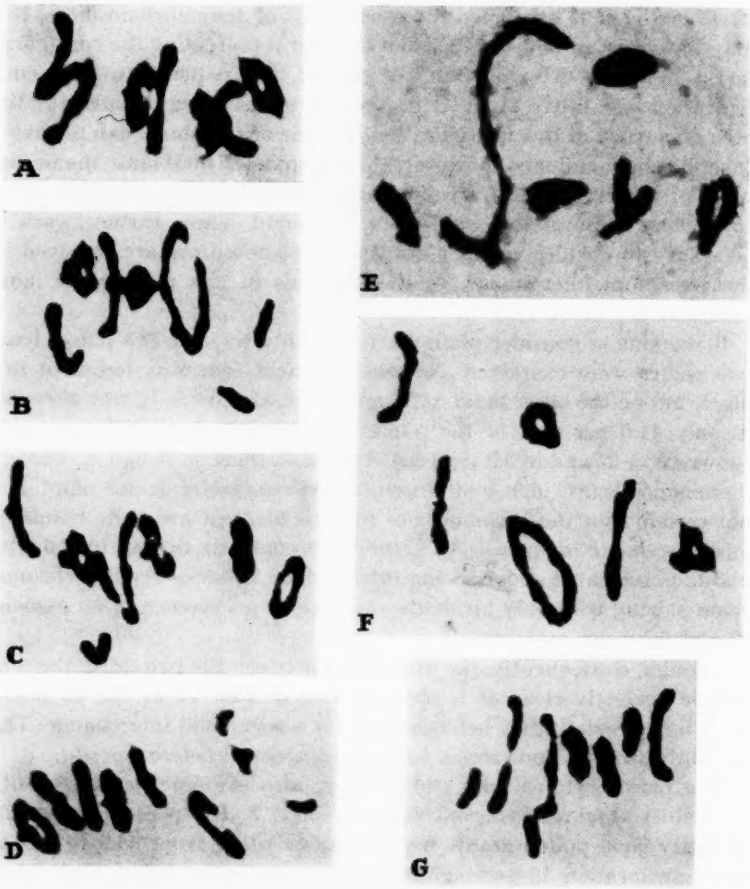

Fig. 1. Chromosome configurations in translocation heterozygotes. — A, Zig-zag chain of four and 5 bivalents; B and F, the association of four represented by a closed, non-zig-zag ring; C, $1_{III}+1_{I}+5_{II}$; D and G, no association of four but instead 7_{II} (D) or $6_{II}+2_{I}$ (G); E, pro-metaphase with $I_{IV}+5_{II}$. — × 2500.

p.m.c. with zig-zag chains or rings at first metaphase ranged from 56.5 to 92.8 with an average of 74.9.

A typical zig-zag chain of four and 5 bivalents are represented in Fig. 1 A. In Figs. 1 B and F the association of four is represented by a closed, non-zig-zag ring, and in Fig. 1 C the corresponding chromo-

somes form a V-shaped trivalent and a univalent. All these p.m.c. are from plant no. 1, just as Fig. 1 E, representing a somewhat earlier stage. Figs. 1 D and G are from plant no. 11, in which the four chromosomes involved in the translocation were often replaced by two bivalents (as in Fig. 1 D) or one bivalent and two univalents (as in Fig. 1 G).

The 25 per cent of the cases in which adjacent chromosomes pass to the same pole at anaphase are sufficient to explain the moderate reduction in pollen fertility actually observed. As there is a rather pronounced variation in pollen fertility as well as in frequency of zig-zag-arrangements the coefficient of correlation was calculated and found to be as large as $+0.72$. A test of significance of this coefficient gave a P-value intermediate between 0.02 and 0.01. Hence, it is highly probable that pollen fertility, in this material is positively correlated with frequency of zig-zag-arrangement at first metaphase.

Table 2 also gives information on the percentages of seed setting in the 13 plants examined. The values are all quite low, but in the first place this is certainly caused by inadequate pollination, the plot being small and isolated and the flowering-time of the different spikes spread out over a relatively long period. Hence, the values may scarcely be used for estimations of various degrees of real sterility. It is noteworthy, however, that the two plants lacking an association of four chromosomes at meiosis both had poor seed setting.

DISCUSSION AND CONCLUSIONS

The chromosomes of *Secale Kuprijanovii* have already been examined by NAKAJIMA (1954) who reported meiosis to be predominantly normal with 7 bivalents. His material was a strain obtained from G. F. MAKKINK, Netherlands. It is very unlikely that the strain studied by us should be related to the strain studied by NAKAJIMA. Thus, different strains of this species [1] evidently have different modes of meiosis. In the present strain the preponderance of individuals heterozygous for a segmental interchange is, indeed, very pronounced, at least 11 of 13 individuals examined representing this condition.

We do not know whether the seed sample obtained from Leningrad was derived from a single individual or from a mixture of individuals. In the former case the mother plant was probably a translocation

[1] The species studied by NAKAJIMA is called *Secale Kukrijanovi*, GROSSH., which undoubtedly corresponds to the species usually spelt *Secale Kuprijanovii*.

heterozygote, which should be expected to segregate translocation heterozygotes and plants with normal meiosis in the ratio 1:1. A χ^2 test of the ratio 11:2, actually found, gives a χ^2 of 6.23 which corresponds to a P-value intermediate between 0.02 and 0.01. If plant no. 7 is excluded, as the observations of meiosis in this plant were limited to diakinesis, the observed ratio would be 11:1. This ratio gives a χ^2-value of 8.33, corresponding to a P intermediate between 0.01 and 0.001. Thus, it is very unlikely that the ratio observed represents a 1:1 ratio. If, on the other hand, the seed sample was derived from more than one individual it is probable that these individuals, or the great majority of them, were translocation heterozygotes.

The present data are not sufficient to clarify in which way the structural heterozygosity in this material is upheld, but probably the heterozygotes are in some way superior to the two classes of homozygotes. Another possibility is, of course, the occurrence of heterogamy.

Previously, significant deviations from the expected 1:1 ratio have also been reported for material of *Secale*. Thus, MÜNTZING and PRAKKEN (1941) found that in the offspring of a spontaneous translocation heterozygote of *Secale cereale* there was a significant *deficit* of translocation heterozygotes. More precisely, of 21 plants raised from seeds obtained after open pollination only 2 were translocation heterozygotes, 19 plants having 7_{II} at meiosis. Of 9 plants raised after isolation 6 had 7_{II}, and 3 were found to have an association of four. On the other hand a certain translocation heterozygote, being a hybrid between cultivated varieties of *Secale cereale* from Sweden and Ecuador, gave a significant *excess* of translocation heterozygotes in the offspring (AKDIK and MÜNTZING, 1949). More work is needed to decide to what extent differential viability of gametes, zygotes or mature plants or differential fertility of the plants may account for the deviations observed.

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INHERITANCE OF THE COLOR PHASES IN RANCH BRED BLUE FOXES

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NATURAL populations of the arctic fox (*Alopex lagopus*) are usually a mixture of two different color phases known as white foxes and blue foxes. The former is completely white in the winter and sooty brown in the summer, whereas the latter never turns white although it may show considerable seasonal changes in color. The proportion between the two color phases shows a great variation between different regions of the circumpolar distribution of the species, and the same is true with regard to the degree of pigmentation of the winter coat of the blue foxes. However, the color phase of the white fox and the blue fox are always quite distinct, and there are no transitions between them.

Two geographical races of the arctic fox have been ranch bred in the Scandinavian countries, viz. the Alaska blue fox and the Greenland blue fox. The Alaska blue fox is a native of the islands and peninsulas along the south-west coast of Alaska. The winter coat is uniformly dark, and when the Alaskan blue foxes are bred *inter se* there is no segregation of white foxes. The Greenland blue foxes vary considerably between regions in the degree of pigmentation, and in mixed populations the blue foxes do not breed true for their color phase. On the basis of dietary habits and geographical distribution BRÆSTRUP (1941) distinguishes between the migratory "lemming fox" and the "coast fox". Within the former the whites are predominating and the blues are rare, whereas the reverse is true with regard to the "coast fox". On some bird cliff islands along the west coast of Greenland there may not be any white foxes at all.

The breeding of Alaska blue foxes in captivity started about the turn of the century, and the first imports to the Scandinavian countries were made in the twenties. An import of light colored blue foxes, mainly from the north-east of Greenland, started about ten years later, and this

type soon became very popular for ranch breeding because of its attractive color and soft fur. One disadvantage was, however, that the Greenland blue foxes, mated *inter se*, segregated 5—10 % white foxes. The constitutional vigor of the segregates was found to be lower under ranch conditions than that of the blue fox, and the pelts had to be sold at a considerably lower price. Nervous disturbances with trembling and convulsions as the main symptoms, known as the "white fox disease", had a high frequency among the white foxes. Crosses were made between the Greenland and Alaska blue foxes in order to improve the bozy size and fertility of the former. In the first cross-bred generation there was no segregation of white foxes, but in later generations, and in backcrosses to the Greenland type, the whites appeared again. Therefore, the ranchers asked for advice how to breed a light colored blue fox which does not segregate whites. This question gave the incitement to the present investigations.

I. DIFFERENCES BETWEEN RANCH-RAISED ALASKAN AND GREENLAND FOXES

The Alaskan blue fox is somewhat larger and more robust than the Greenland fox, which has a shorter body and a finer skeleton. The Greenland foxes put on more fat in the fall and early winter which makes them appear short and plump compared to the other type. The average weight of young Alaskan and Greenland foxes at pelting time is therefore not much different. The breeding season of the Alaskan blue fox starts about a week later than that of the Greenland foxes. The litter size is larger; on an average 10 young at birth as compared to 8.5 for the Greenland type.

The Greenland blue fox has a much thicker underfur and shorter and finer guard hairs than the Alaskan fox which makes the whole fur more soft and silky. BRÆSTRUP (1941) quotes a statement by an other observer according to which the white foxes in mixed populations have longer guard hairs than the blue ones. BERGE *et al.* (1956) found that the white fibers of the underfur of Greenland blue foxes were almost twice as long, on an average, as the pigmented fibers when the winter coat was fully developed.

The "bluish" color of the Greenland blue foxes results from a mixture of white and pigmented hairs. As a rule, the color of the single wool hairs and guard hairs is the same throughout their entire length, although some guard hairs may have black tips. The banded pigmenta-

tion of the guard hairs, which is typical for the red fox and the silver fox, does not exist in blue foxes. The shade of color of the Greenland blue foxes depends on the proportion between white and pigmented hairs. Very light colored foxes may not have more than 25 % pigmented hairs (BERGE *et al.*, 1956). — The general impression of the fully developed winter coat of the white fox is a clear white color. By closer inspection a few white guard hairs with black tips may be discovered, and quite many pigmented wool hairs are always found.

The winter coat of the Alaska blue fox has long guard hairs and not very dense underfur. There is no noticeable admixture of white hairs; the coat color is mainly determined by the intensity of pigmentation of the guard hairs. The Alaskan blue fox is considerably darker than even the darker shades of Greenland blue foxes.

II. SEASONAL COLOR CHANGES

The whelping season extends from the middle of April to the beginning of June; about 80 % of the births occur between the first and the 20th of May. The one year vixens whelp about a week later than those in higher age groups.

The young are born with hairs and during the first week of postnatal life there is a rapid development of underfur and guard hairs. At this stage it is not possible to distinguish clearly between white and blue foxes because they show the same degree of pigmentation. However, when the white fox whelps are two or three weeks old, cream colored guard hairs begin to appear on the ventral side of the body. These hairs increase in number, and when the whelps are 4—5 weeks old they can be sorted according to color phase without any difficulty (Fig. 1). At this age the whelps experience their first molt; the guard hairs are gradually replaced by new ones which are only slightly longer than the underfur. The result is the typical summer coat which along the dorsal side is rather alike in the Greenland blue and white foxes as well as in the Alaskan blue fox. The white fox, however, is considerably lighter (yellowish) on the ventral side (Fig. 2 A).

The transition of summer coat to winter coat (the second molt) starts early in September. Most of the summer guard hairs and wool hairs are shed, and new hairs appear in increasing number (Fig. 2 B) and grow to almost twice the length of the summer hairs (SKÅRMAN, 1953). This growth continues until December when the winter fur reaches maximum of development. The guard hairs and most of the wool hairs

Fig. 1. Four weeks old cubs from a mixed litter: a blue fox to the right and a white fox to the left. The cream colored guard hairs along the ventral side of the white fox cub reveals the genetic identity of the animal.

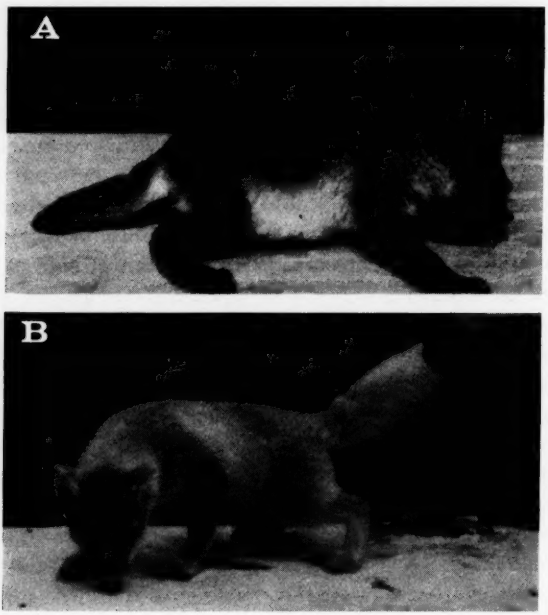

Fig. 2. Seasonal color changes in the young white foxes. — A. A six weeks old white fox cub with typical summer coat (July). B. A four months old white fox cub changing to winter coat (September).

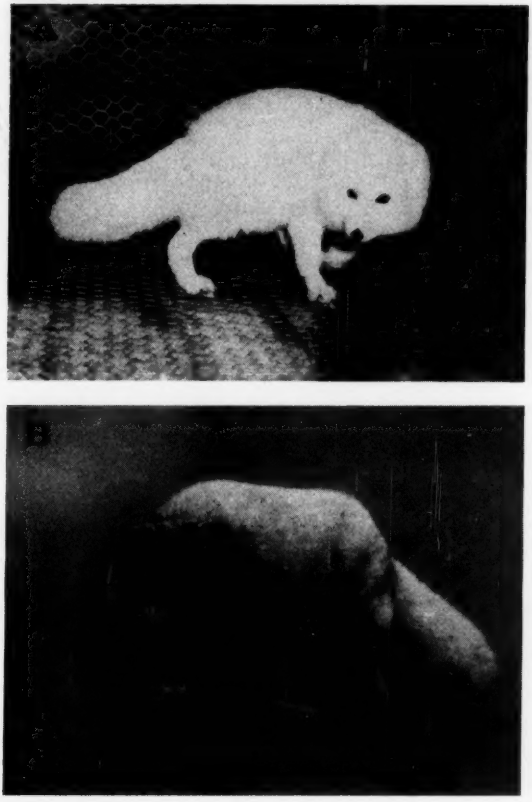

Fig. 3. Winter coat of ranch bred Greenland foxes (December—January). — A. White fox. B. Greenland blue fox with a mixture of pigmented and white hairs.

of the white fox are then entirely white, whereas the Greenland blue fox has a mixture of dark and white hairs (Fig. 3 A and B).

The young cubs of the Alaskan blue fox are darker than those of the Greenland blue fox, but otherwise the difference in color is slight until the second molt in September. The color of the winter coat is bluish brown, and it differs from the summer coat mainly by the higher intensity of pigmentation of the guard hairs.

In the mature animals the spring molt starts in March—April on head and legs, and proceeds along the dorsal side of the body. On the ventral side of body and tail the winter guard hairs are rather per-

Fig. 4. A mature white fox in summer coat (July).

sistent. Fig. 4 shows a mature white fox after the spring molt (beginning of July); some of the white guard hairs of the winter coat are still left on tail and belly. The fall molt starts in September, and the winter coat develops gradually until November—December. The color changes during the fall molt are identical with those in the young animals.

III. INHERITANCE OF THE COLOR PHASES

1. Statistical analysis of data from blue fox ranches

Data accumulated from 104 Swedish ranches were analyzed with regard to segregation of the white color phase in matings blue×blue as well as the variations in shade of color among the Greenland blue foxes.

The material included 234 litters of the Alaskan blue fox with 2,328 young at the time of counting; usually within a few days after birth. In these litters there was no segregation of white foxes, and no appearance of any whelps of the bluish-grey Greenland type. The Alaskan foxes breed true for their color phase.

The data for the Greenland type of blue foxes were divided into two groups on the basis of the ancestry of the animals. One group consisted of litters where both parents were of "pure" Greenland descent, according to information from the breeders, and one group where one or both parents were the result of crossbreeding between the Greenland and the Alaskan types. There are probably some errors in this classification, because the breeders did not always know about crossbreeding that had been carried out farther back in the ancestry of the parents, but it was the best that could be done under the circumstances. The definite classification of the young into color phases, *i.e.* blue and white foxes, was usually done at marking time, when the whelps were about two months old. The mortality in the litters up to this time is about

15 %, on an average, most of which occur during the first week after birth. There was no possibility to find out if this mortality was differential with regard to the color phases, since these can not be distinguished before one month of age.

In the group of "pure" Greenland foxes records were available for 762 litters with 6,234 young of which 405 were classified as white foxes, or 6.50 % of the total number. All these litters were obtained from matings of blue fox×blue fox; the white foxes are rarely used for breeding. Assuming that the white-fox gene is completely recessive, and that therefore selection against this gene was based only on the white segregates, which is not quite true but may be used as an approximation, and denoting the frequency of the white-fox gene in the total population with q, the frequency of the heterozygotes in the breeding population containing no whites would be

$$\frac{2q(1-q)}{(1-q)^2+2q(1-q)}=\frac{2q}{1+q}.$$

The frequency of the recessive gene for the white color phase within the same population would then be $\frac{q}{1+q}$. This expression should be equal to $\sqrt{0.065}=0.255$, and q for the whole population, including the whites, would be 0.342. — The other group of more or less crossbred parents of the blue fox type produced 169 white foxes out of 6,055 young in 696 litters, *i.e.* 2.79 % white foxes corresponding to a gene frequency among the "blue" parents of 0.167. Thus, the use of Alaskan blue foxes for crossbreeding had materially decreased the frequency of the recessive gene and the segregation of white foxes. — No cases were reported where the Alaskan color type segregated out when crossbred animals were mated *inter se*.

A few breeders had carried out matings between Greenland blue foxes and white foxes. Thirty litters obtained from such matings contained 257 young of which 68, or 26.5 % were white. If the frequency of the white-fox gene had been the same among the blue parents as previously estimated for the Greenland blue fox population, 25.5 % whites would have been expected, which is a good agreement to what was actually found.

On one ranch a study was made of the segregation of white foxes when known heterozygotes were mated to one another, *i.e.* when both parents in previous litters (not included) had thrown white foxes. In 117 litters 194 whelps out of 1010 were white, or 19.2 %. This is a signi-

ficantly lower ratio than would be expected from matings between heterozygotes (S.D.=1.24). Differential mortality is perhaps the most likely reason. For the total material it was calculated that the average size of 856 litters in which no white whelps were found, was 8.73, whereas the average size of 376 litters with one or more white whelps was only 7.84. This seems to indicate a higher mortality for the white than for the blue color phase.

The percentage of white whelps in the 376 litters mentioned above was 24.8 % which is very close to the 3 : 1 ratio. It must be remembered, however, that matings between heterozygotes, where no white whelps were born, or had survived until the time of classification, are not included, and that the calculated ratio therefore is deficient. A correction for the exclusions could be made (HALDANE, 1932) but correction for the differential mortality in litters with one or more white whelps is impossible. Therefore, the figures are presented without any further comments.

On one large Swedish ranch of Greenland blue foxes a system of scoring each animal for its color had been practiced during several years, and the records were placed at our disposal for analysis. An elaborate system of letters was used to indicate the different shades of color of the coat at pelting time as well as the estimated frequency of white guard hairs ("silvering"). These letters were transformed to figures (1—24), the lowest figures denoting the darkest and the highest figures the lightest color types. No females with lower score than 7 had been used for breeding, and practically all breeding males had very high scores. Since there was little variation between the males, only the results of a study of the relation between the color scores of the dams and that of their offspring is presented here (Table 1).

TABLE 1. *Relation between color of dams and that of their offspring.*
All males had color scores above 18.

Dams		Offspring				
Color group	Scores	Blue	White	Total no.	% White whelps	Av. score of blues
Dark silvered	7—12	482	20	502	4.0	16.1
Medium light	13—17	1636	113	1749	6.5	14.3
Light	18—21	3341	268	3609	7.4	21.2
Extra light	22—24	1944	185	2129	8.7	17.5
Total and average		7403	586	7989	7.3	18.4

The frequency of white segregates increases significantly ($\chi^2=15.1$ and $P<0.002$) with decreasing pigmentation of the dams. There is a slight indication also of decreasing pigmentation of the blue offspring with decreasing pigmentation of the dams but the trend is barely significant.

2. Breeding experiments

A breeding experiment with blue and white foxes was carried out under three successive years at the Experimental Fur Ranch of the Royal Agricultural College. The start was made with 2 males and 12 females of white foxes, and 2 males of Greenland blue fox which both had thrown white-foxes, and thereby proved themselves to be heterozygous for the white character. The second year, males of the Alaskan blue fox were used for crossbreeding with white vixens, and the third year 10 F_1 females from this cross were back-crossed to white males. The reproductive records of the white vixens were much poorer than those of the F_1 females or Greenland blue fox vixens. Two white vixens and one white male died in the "white fox disease", and several of the other white animals showed more or less pronounced symptoms of the same disturbance. Two whole litters from white mothers were eaten the first day after birth, before counting. On an average, 11.4 % of the counted young died before they could be classified according to their color phase. The experimental results with regard to the classified young are summarized in Table 2.

TABLE 2. *Result of the crossbreeding experiments.*

Mating	Number of litters	Number of classified whelps	White	Blue
White×white	5	36	36	—
Heterozygous blue ♂♂×white ♀♀	4	40	19	21
Alaskan bluefox ♂♂×white ♀♀	4	26	—	26
Backcross: F_1 ♀♀×white ♂♂	8	70	37	33

Matings white×white produced only white foxes. The same results have been obtained by SLAGSVOLD (1949) in 8 experimental litters with a total of 59 young. When the heterozygous Greenland blue fox males were mated to white vixens, about equal number of blue and white whelps were obtained, but when Alaskan males were used on white

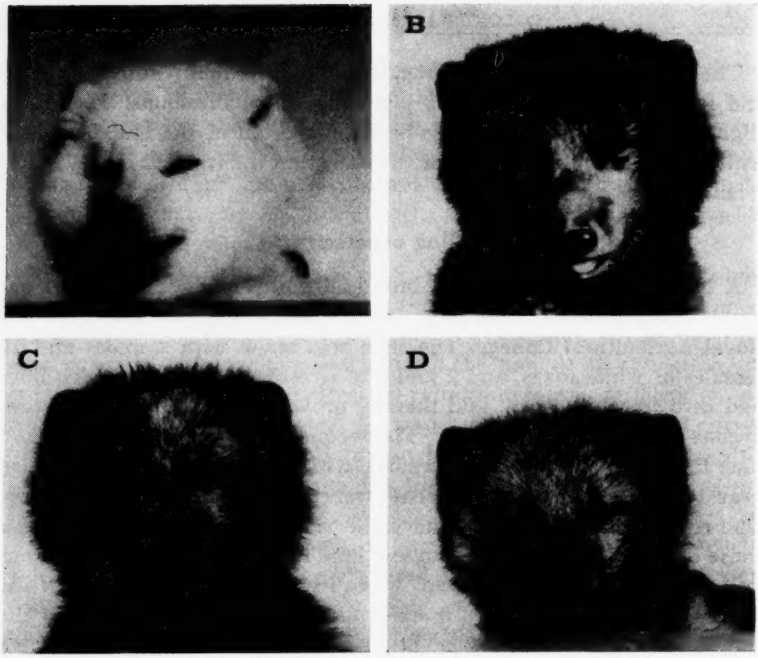

Fig. 5. Head pictures of four typical experimental animals. — A, white fox; B, Alaskan blue fox; C, F_1 from the cross between Alaskan and white fox; D, Greenland blue fox.

Fig. 6. Pelts of white fox and Alaskan blue fox.

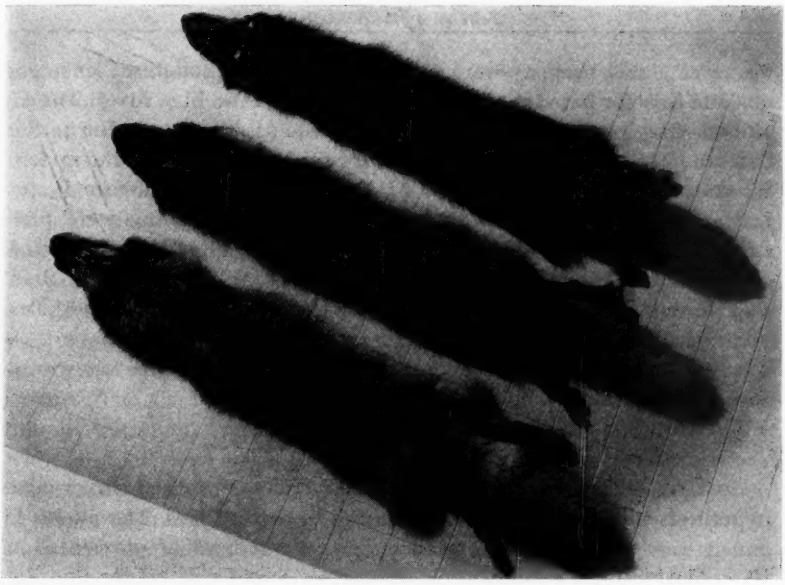

Fig. 7. Pelts of F_1 animals from the cross between Alaskan and white fox showing the variation in shade of color and length of guard hairs.

Fig. 8. Typical pelts of Greenland blue foxes.

vixens all the F_1 progeny were of the "blue" type, although rather intermediate in color between the Alaskan and Greenland blue foxes. The F_1 animals had longer guard hairs but not quite as dense underfur as the Greenland foxes. White guard hairs were fairly frequent and the underfur was lighter than that of the Alaskans. In matings between F_1 females and white males 8 litters with 70 classified young were produced, 37 white and 33 blue whelps which is fairly close to the expected 1 : 1 ratio. On an average, the blue segregates were lighter in color than their F_1 dams, and the white segregates had plenty of white guard hairs with black tips, apparently a heritage from the Alaskan grandparent. — Typical pelts and heads of the experimental groups are shown in Figs. 5—8.

IV. DISCUSSION AND CONCLUSIONS

BOITSOV (1937, reviewed by BACKHOFF, 1939) proposed a two gene hypothesis to explain the color variation in arctic foxes. The alleles D and d were supposed to determine the general level of pigmentation (D — dark, and d — light), and the alleles S and s stand for the pigmentation of the individual hairs (S — uniform color, and s — "silvering"). The white foxes were assumed to be double recessives, ddss, the "silvered" would be D—ss, the "blue" ddS—, and the dark D—S—. However, this hypothesis does not fit the facts which are presented in this paper and by SLAGSVOLD (1949). When proven heterozygotes of silvered Greenland blue foxes are mated together, the proportion between the colored and the white offspring is so close to the 3 : 1 ratio, that there are no reasons to suspect that the gene, or genes, for "silvering" has anything to do with the white fox character. SLAGSVOLD (1949) concluded that the white color phase in the Greenland arctic fox is determined by an autosomal recessive gene, and the results from our own investigations support this conclusion. However, the recessivity of the gene for white is not complete. Table 1 shows that the frequency of white fox segregates increases with decreasing pigmentation of the dam when blue Greenland vixens are mated to light blue-fox males. Furthermore, the F_1 animals from crosses between Alaskan and white foxes are always lighter in color than their Alaskan parent, although there is a variation between them in the degree of pigmentation.

The Alaskan blue fox which has been used for breeding in Sweden, does not carry the gene for the white color phase. The variation in color of the F_1 animals mentioned above is probably due to polygenes for shades of color which genes were carried by the white parents. Cross-

breeding results reported by LEEKLEY (1943) agree with this interpretation.

Many Greenland blue foxes never segregate white foxes even when they produce 30—50 offspring in their life time after matings to light colored males, but in spite thereof they vary in the degree of pigmentation. The frequency of wholly white hairs (the "silvering") varies and there seems to be also a concurrent variation in the color intensity of the pigmented hairs. Therefore, it may be concluded that the variation in "silvering" and general shade of color is a quantitative character which is influenced by many genes. In addition, the gene for the white fox character has a certain lightening effect on the color of the heterozygotes. The shade of color of the Greenland blue foxes shows a continuous variation from dark to light, but the difference between the white and the blue color phase is always distinct without transitions between them. This is true also when white-foxes segregate after a cross to the Alaskan blue fox.

Apparently, there are good possibilities of eliminating the gene for the white color phase from a population of the Greenland blue fox by selection. The most rapid way would be to progeny test all one year old males by mating them to white vixens; only one or two litters reaching weaning age would be required for this test. Then only males which have not thrown white foxes in such matings should be used to cover blue-fox vixens. It is an open question whether or not it would be possible to produce the lightest shades of Greenland blue foxes after elimination of the gene for the white color phase. However, it would certainly be possible to produce rather light shades by the accumulation of polygenes for "silvering" and a low degree of pigmentation.

SUMMARY

A study has been made on the color inheritance in ranch-bred Greenland arctic foxes as well as in crosses between white foxes and the Alaskan blue fox. Data from 104 Swedish blue fox ranches were analysed, and in addition breeding experiments were carried out on a small scale.

The results of the investigations can best be explained on the assumption that the white color phase of the Greenland foxes is determined by an incompletely recessive gene which has a certain lightening effect on the color of the heterozygotes. However, most of the variation in the blue color phase depends on polygenes affecting the frequency of white

hairs in the coat ("silvering") as well as the color intensity of the pigmented hairs.

The Alaskan blue fox, which has been ranch bred in Sweden, does not carry the recessive gene for the white color phase and has practically no polygenes for "silvering". F_1 animals from crosses between Alaskan and white foxes are intermediate in color to their parents. Polygenes for "silvering" are introduced into this cross by the white foxes, and in addition the white-fox gene decreases, to some extent, the pigmentation of the heterozygotes.

It should be possible to eliminate the white-fox gene from the Greenland blue foxes by selection without losing much of their pleasing light bluish-grey appearance, because polygenes for decreased pigmentation can be accumulated according to the wish of the breeder.

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BRIEF REPORTS

D. VON WETTSTEIN: Multiple allelism in induced chlorophyll mutants of barley.

(Received January 20th, 1960)

Chlorophyll mutants have been useful in studying the effect of genes on the submicroscopic structure of the chloroplast and in analyzing the genetic control of the formation of macromolecular cell structures (v. WETTSTEIN, 1957/59, 1958, 1959). One would like to test the current concepts of the physiology of gene-action from a structural point of view at the submicroscopic level employing different alleles of the same gene. Only two cases of allelism in chlorophyll mutants of barley seem to be known to date: the spontaneous mutants *xantha*-2 and *xantha*-3 were allelic (NILSSON-EHLE, unpubl.; GUSTAFSSON, 1940). Unfortunately, *xantha*-2 has been lost. In chromosome 2 (former linkage group I) a gene for *virescens* seedlings with two different alleles (y and y^x) — both spontaneous in origin — has been localized (ROBERTSON and COLEMAN, 1942; HAGBERG and FAVRET, 1960). Even in such a well analyzed plant like maize, only scattered cases of allelism among chlorophyll mutants were known until recently (EMERSON, BEADLE and FRASER, 1935). ROBERTSON (1958, 1959) tested 16 different albino and pale green mutants for allelism. They belong to nine different loci, and multiple allelism was found in four of them. Thus, four alleles are known in locus vp-5, three in locus w-3, and two each in the loci lw-2 and w 8624. In our assortment of about 100 different chlorophyll mutants induced by ionizing radiation or chemical mutagens, extensive crossing has been carried out during the last three years in order to find allelic relationships. The efforts were especially concentrated on the *xantha*-types since these seem to be the most interesting mutants for submicroscopic analysis.

So far it has been found that *xantha*-12, *xantha*-15 and *xantha*-18 are allelic to each other, whereas they proved to be non-allelic to xa-10, xa-11, xa-13, xa-14, xa-19 and xa-20, which all seem to represent different loci.

Some data about the origin and segregation of the lethal mutants xa-12, xa-15 and xa-18 are gathered in Table 1. All three behave as single factor mutants segregating in a 3 : 1 ratio. Xa-15 arose simultaneously with a translocation, which however is not linked to that locus. Similarily an unlinked male-sterility mutant was obtained from the same spike progeny as xa-18. Plants heterozygous for one of the three *xantha*-alleles are normal green and fully fertile. Phenotypically, the three mutants are variable, their colour depending on the temperature and light conditions under which they are grown. This is reflected by the Code de Coleur numbers assigned to the mutants in greenhouse tests during different years (lower part of Table 1). Under certain conditions (1958) they cannot be distinguished from each other by their leaf colour. If differences in leaf colour occur, the alleles can be seriated according to increasing chlorophyll content, whereby xa-12 consistently has the lowest amount of pigment and xa-18 the highest. In certain years xa-15 and xa-18 would be classified as *viridis* instead of *xantha*.

The crosses from 1959 between the three mutants which proved the allelic relationships are summarized in Table 2. 178 crosses were carried out and

TABLE 1. *Data on the allelic mutants xantha-12, xantha-15 and xantha-18.*

	xa-12	xa-15	xa-18
Mother line	*Erectoides* 23	*Erectoides* 23	*Erectoides* 23
Year of origin	1954	1954	1954
Mutagen	neutrons, 250 rad	neutrons, 500 rad	x-rays, 5 200 rad
Segregation (1959)			
observed:	2147 : 712 xa	2059 : 698 xa	1627 : 549 xa
expected:	2144.25 : 714.75 xa	2067.75 : 689.25 xa	1632 : 544 xa
	$\chi^2 = 0.014$; P~0.9	$\chi^2 = 0.148$; P~0.7	$\chi^2 = 0.061$; P~0.8
Code de couleur Nr. (green-house) (Klincksieck et Valette, 1908)			
1954	241	241	246 with green tip
1957	246	261	281
1958	216	216	216
1959	221	246	266 with green tip
	216—247 = different shades of yellow 261, 266 = yellow-green 281 = light-green		

seed-set was obtained on 137 spikes (77 %). In agreement with the expectation, 69 F_1 spike progenies resulted from crosses between plants heterozygous for *xantha*. The segregation of the F_1 in all possible combinations between the mutants shows good agreement with a 3 : 1 ratio. In addition the following remarks should be made:

1) Of the 69 F_1-spike progenies, nine did not segregate for *xantha*, their average number of kernels per spike being 5,4. Such non-segregating ears are expected in small progenies.

2) Because of technical difficulties in carrying out crosses of this kind on a large scale, a certain degree of self-pollination in some of the spikes could not be avoided. The amount of selfing can be estimated from crosses where only the mother plant is heterozygous. In the combination xa-18 × xa-12 25 % of the spikes from heterozygous plants pollinated with wild-type pollen revealed self-pollination; in the other combinations no unsuccessful crosses of this type were found. The phenotype of the *xantha* plants obtained from crosses between heterozygotes allows classification of illegitimate selfing in four of the combinations. Among these 16 % of the spikes showed phenotypes which may be accounted for by selfing. Consequently, only about 20 % of the 189 *xantha* seedlings found in the F_1 spike progenies could possibly be explained by self-pollination.

3) The allelism is clearly shown by the phenotype of the seedlings. The leaf

TABLE 2. *Results of crosses between xa-12, xa-15 and xa-18.*

Combination	♀ Green : *xantha*	♂ Green : *xantha*	F_1				
			Number of spikes	Green : *xantha*	χ^2	P	Phenotype of *xantha*
xa-12×xa-15							
observed	790 : 245	691 : 226	20	149 : 53			Lighter in colour
expected	776.25: 258.75	687.75: 229.25		151.50: 50.50	0.165	0.7	than both parents
xa-15×xa-12							
observed	235 : 80	231 : 81	7	43 : 11			Lighter in colour
expected	236.25: 78.75	234 : 78		40.50: 13.50	0.617	0.4	than both parents
xa-12×xa-18							
observed	408 : 131	372 : 133	12	93 : 34			Like the mother (xa-12)
expected	404.25: 134.75	378.75: 126.25		95.25: 31.75	0.155	0.7	
xa-18×xa-12							
observed	443 : 133	278 : 103	11	102 : 40			Like the father (xa-12)
expected	432 : 144	285.75: 95.25		106.50: 35.50	0.661	0.5	
xa-15×xa-18							
observed	260 : 102	261 : 85	8	44 : 23			Like the mother (xa-15)
expected	271.50: 90.50	259.50: 86.50		50.25: 16.75	3.110	0,1 > P > 0.05	
xa-18×xa-15							
observed	405 : 153	280 : 83	11	84 : 28			Like the father (xa-15)
expected	418.50: 139.50	272.25: 90.75		84 : 28	0	1	
		Total:			Σ 4.708	0.6 (6 d.f.)	
		observed	69	515 : 189			
		expected		528 : 176	1.280	0.3	
		Heterogeneity			3.428	0.6 (5 d.f.)	

colour of the seedlings heterozygous for xa-12 and xa-15 is lighter than both parents. The phenotype of xa-12/xa-18 is indistinguishable from xa-12/xa-12 and that of xa-15/xa-18 from xa-15/xa-15. Thus, in the genotypes xa-12/xa-18 and xa-15/xa-18 the "lighter" allele is dominant over the "darker". In the combinations where the allele causing a lower amount of pigment is used as the pollen parent, the F_1 *xantha* seedlings showed the same phenotype as their father.

Electron-microscopical studies of these mutants revealed that their chloroplasts are able to form some lamellae. These lamellae are, however, not of the normal type and aggregate in a wrong fashion. It is of interest to note that all three allelic mutants are blocked at the same stage in plastid development and that all three produce the same aberrant lamellar system in their plastids. Further electron-microscopical and biochemical studies are in progress.

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ALBERT LEVAN and T. C. HSU: The chromosomes of a mongoloid female, mother of a normal boy.

(Received July 25th, 1960)

The present writers recently published in this journal a human idiogram, based on ten karyotype analyses of a ten year old boy, the child of a mongoloid mother (LEVAN and HSU, 1959). It was mentioned at that time that the karyotype of the mother would be studied and reported on later.

Fig. 1. The chromosomes of the mongoloid female. — × 3300.

A skin biopsy, taken from the mongoloid mother in April, 1960, has yielded vigorously growing cultures. Slides from a second subculture, prepared according to HSU and KELLOGG (1960), contained many easily analyzable mitoses. The chromosomes of the mongoloid mother are represented in Fig. 1. The karyotype is that typical of female mongoloids. The chromosome number is 47 and five small chromosomes of types 21 and 22 are present instead of the four chromosomes of these types characteristic of normal females. Just as was found in the karyotype of the son, the acrocentric chromosomes 13—15 of the mother had more pronounced heads than usually seen in these chromosomes. This is probably due to the technique which seems to emphasize short chromosome segments.

Among some hundred metaphases examined, one deviated by having 48 elements. This was due to a break in the longer arm of one of the group 6—12 chromosomes. Out of five complete karyotype analyses, four were perfectly normal, while one indicated a probable translocation between two chromosomes of the 6—12 group. Only 14 typical chromosomes of this group were present and, in addition, one larger chromosome, similar to type 3, and one smaller chromosome, approaching type 16. If this indication of an increased incidence of structural changes is significant, it should be looked into whether the genetic imbalance caused by the trisomic condition may predispose to

chromosome breakage. Certain results by REITALU (unpublished) in Klinefelter patients go in this direction.

It may be concluded from the present investigation that a normal 23 chromosome gamete of the mongoloid mother has functioned at the conception of the normal child. This is in agreement with experiences from plants, where crosses between diploids and trisomics usually give progenies with a majority of diploids and some trisomics.

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TILL MEDARBETARNA

Manuskript — helst *maskinskrivna* — torde insändas till redaktionen (Genetiska Institutionen, Lund) i fullt tryckfärdigt skick. De böra vara *noga genomsedda* för undvikande av ändringar mot manuskriptet. Obs. kommateringen! Korrektionskostnaderna betalas av författaren. Korrektur ställes till redaktionen. Direkt förbindelse mellan författaren och tryckeriet tillåtes icke.

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